PAPER SPRAY MASS SPECTROMETRY FOR RAPID DRUG SCREENING

by

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To the Creator-
Who places the mysteries of the world before us
and the curiosities of the mind within us

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Thank you.

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LIST OF ABBREVIATIONS

AFT	Axis Forensic Toxicology
CE	Collision Energies
CID	Collision Induced Dissociation
ELISA	Enzyme-linked Immunosorbent Assays
EMIT	Enzyme-multiplied Immunoassay
ESI	Technique Electro Spray Ionization
FPIA	Fluorescence Polarization Immunoassays
GC-MS	Gas Chromatography-Mass Spectrometry
GUS	General Unknown Screening
HPLC-MS	High Performance Liquid Chromatography Mass Spectrometry
HR-MS	High Resolution Mass Spectrometry
IS	Internal Standards
KIMS	Kinetic Interaction of Microparticles in Solution
LC-UV	Liquid chromatography with Ultra Violet Detection
LLE	Liquid-Liquid Extraction
LLOQ	Lower Limit of Quantitation
LOD	Limit of Detection
MTS	Multi-Target Screening
PMR	Post-mortem Redistribution
PS	Paper Spray
PS-MS	Paper Spray Mass Spectrometry
QqQ	Triple Quadrupole Mass Spectrometers
RIA	Radioimmunoassays
S:B	Signal to Blank Ratio
SRM	Selected Reaction Monitoring
STA	Systematic Toxicological Analysis
WADA	World Anti-Doping Agency
XIC	Extracted Ion Chronograms

ABSTRACT

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Paper spray mass spectrometry is an alternative technique for toxicological screening that is able to quickly and adequately screen for compounds encountered in postmortem investigations with little sample handling and no sample preparation. For analysis of dried blood spots using a triple quadrupole mass spectrometer, detection criteria were defined to align with relevant regulatory guidelines while considering how fragment ion selection, method sensitivity, and fragment ion ratio tolerances are best utilized in paper spray mass spectrometry. For analysis, drugs and drug metabolites relevant to postmortem investigations were spiked into drug-free blood, and by monitoring two fragment ion channels in selected reaction monitoring mode, as well as the ratio between the two fragment ions, a method was developed capable of detecting over 120 drug and drug metabolites at concentrations relevant to postmortem drug screening. Total analysis time for the developed method is less than 8 minutes, and less than 50µL of sample and 5mL of solvent are consumed during analysis.

1 BACKGROUND

The following project was undertaken with the goal of developing a multi-target toxicological screening process that would be applicable for use in forensic postmortem investigation cases. While the same method may be applied in different areas of forensics or even in different fields within toxicology, it was postmortem investigation that provided the context that shaped this project. As such, a brief overview of how postmortem toxicology relates to other disciplines and how analysis is typically carried out within this context will provide a basis for understanding how this project advances the field of forensic toxicology and why certain decisions were made along the way.

1.1 Toxicology

The word, "toxicology" is derived from the Greek root *toxicos*, meaning "poisonous." At its core, toxicology is simply the study of poisons. Hundreds of years ago, however, one of the fathers of toxicology, Paracelsus, famously stated that any physical or chemical agent can be defined as a poison, and that, "Solely the dose determines that a thing is not a poison."[4] Toxicology then, is actually a very broad field that draws from organic and analytical chemistry, genetics, biochemistry, ecology, pharmacology, pathology, physiology, and other areas of study in order to identify what physical agents and chemical substances cause toxic effects in living organisms, at what dose those effects arise, the mechanisms through which those effects take place, and how to limit or treat exposure to these poisons. The diverse background necessary to understand the complexities of toxicology also allow for its application into a wide variety of situations. From determining how many aspirin we take for headache relief to what chemicals OSHA allows us to handle at work, the impact of toxicology is woven throughout our daily lives.

Although there are a wide range of applications for toxicology, a few sub-disciplines represent much of the prominent work in the field. Clinical and medicinal toxicology are concerned with the development and safe use of medicines and pharmaceuticals that are used to treat, manage, or prevent disease. Environmental

toxicology looks at how industrial waste and byproducts can adversely affect humans and other living creatures in the environment. Food toxicology promotes food safety by monitoring and setting limitations on what pesticides and/or veterinary medicines are safe for human consumption. Forensic toxicology is used when toxicological effects may have legal ramifications, and can include anything from determining a person's cause of death to detecting the use of illegal performance-enhancing drugs in racehorses.

1.2 Forensic Toxicology

While other branches of toxicology are concerned with why a compound is toxic or the mechanisms by which it affects an organism, forensic toxicology is used mostly in situations where the presence or absence of a substance has legal implications. While forensic toxicologists must still understand the hows and whys of toxicology, their primary focus is on the detection, identification, and quantification of toxic agents in the body. There are four essential disciplines within forensic toxicology: human performance investigation, doping control, workplace drug testing, and postmortem investigations.[9]

1.2.1 Human Performance Toxicology

Human performance toxicology is concerned with identifying the presence and concentration of drugs and chemicals which are known to affect the way a person reasons and behaves. Identifying the presence of these chemicals can aid in different types of investigations ranging from collision investigations where a driver is suspected to have been impaired, to drug facilitated crimes such sexual assault cases, and even to child welfare cases. The primary analytical targets of these investigations tend to be alcohol and drugs of abuse.

1.2.2 Doping Control

In competitive sports, using certain performance enhancing substances is illegal and is regulated and monitored by organizations like the World Anti-Doping Agency

(WADA). While most of these drugs have legitimate medical application, banning their use provides a level playing field for athletes and protects their health. These substances, which include steroids, diuretics, and stimulants, are generally not illegal for the general public, but because of the rules and regulations of the professional sports associations, detecting them in athletes can have legal consequences.[9]

1.2.3 Workplace Drug Testing

In 1988, Congress passed the Drug-Free Workplace Act that mandates that federal employees or employees of a company operating on federal money are prohibited from using recreational drugs. Many other companies and organizations, especially those where workers preform potentially dangerous tasks, have chosen to comply with these standards in order to promote workplace safety. Before being hired and during random screenings, employees will give a urine sample which is typically screened for five major classes of abused drugs.[9] If a toxicologist identifies the presence of one of these drugs, there can be occupational and legal consequences for the parties involved.

1.2.4 Postmortem Investigations

The toxicity of a chemical can produce adverse effects ranging anywhere from a headache or a rash to loss of fine motor skills or blindness. In the most severe cases, however, the toxicity of a substance or the dose administered is so extreme that it results in coma or death. In death investigation cases, also known as postmortem investigations, toxicologists work with medical examiners and coroners to help determine cause and manner of death. Unlike in other areas of toxicology, toxicologists investigating postmortem cases must consider a wide range of compounds instead of a small, targeted list. In these cases, toxicologists are presented with a sample that is truly unknown and is presented with the difficult job of detecting, identifying, and quantifying any and all possible toxicants present within any given sample.

1.3 Analytical Strategy

The analytical strategies employed by a forensic toxicologist are often dictated by practicality rather than ideology. Legal goals, limitations within the laboratory, and the circumstances surrounding a case all shape how each sample is processed. Individual labs determine an analytical strategy that is fit for the purpose of each case and is an effective use of the time, money, and resources available to it. In theory, there are two basic analytical strategies: targeted analysis and general unknown screening. In practice, however, many laboratories operate on a hybrid of these methods and use a two-fold method of screening and confirmatory testing.

1.3.1 Targeted Analysis

In cases where there are a finite number of compounds of interest, such as in doping control or workplace drug testing, a targeted, or directed analytical approach may be used. The result of this type of analysis is a list of compounds whose presence in a sample is either confirmed or excluded at certain concentrations. The list of targets analyzed can range from a handful of compounds to several hundred compounds.

Targeted analysis is widely employed in forensic laboratories for a number of reasons. First, forensic toxicology is not an isolated science; it occurs in context. There is often non-analytical information that can serve as a jumping off point for the toxicological examination. If a known drug abuser's body is found surrounded by paraphernalia, a lot of time and money can be saved by first testing for the abused drugs suggested by the investigation. Another reason that targeted analysis is widely used is that a very small number of compounds are typically responsible for death in the majority of postmortem cases. One laboratory reported that 70% of fatal drug poisoning cases in their lab were the result of the less than 30 individual drugs.[10]

Even though only a few dozen compounds are commonly encountered in typical forensic toxicology cases, the list of potential toxic substances is practically limitless. Additionally, new designer drugs are continually developing, and toxicology laboratories are responsible for detecting them, even if it is a new drug that has never been seen before. Because of this, toxicology laboratories cannot solely rely on targeted analytical

techniques, and must incorporate methods to detect a broad range of compounds that are either new or not commonly encountered in typical case work.

1.3.2 General Unknown Screening

The alternative analytical approach to targeted analysis is untargeted analysis, also known as general unknown screening (GUS). The daunting task of untargeted analysis is to identify any and all potentially harmful substances, even when their identity is unknown and their presence is uncertain[11]. In order to provide a framework for untargeted analysis, toxicologists utilize what is known as systematic toxicological analysis (STA), as a guide through the analytical process.

The International Association of Forensic Toxicologists' Committee of Systematic Toxicological Analysis defines systematic toxicological analysis as, "the application of an adequate analytical strategy for the detection and identification of as many as possible potentially toxic compounds and their metabolites in biological samples."[12] The goal of STA is to use a panel of analytical techniques to detect, identify, and if necessary, quantify a very broad range of targets. A few decades ago, this approach meant throwing a sample through every test the lab was capable of because techniques were not as selective or sensitive as they are today.[13]

Modern advances in instrumentation and computational software are opening doors to make general unknown screening a much more viable option. In mass spectrometry, data-dependent, or information-dependent acquisition modes allow for adaptive data acquisition. Developments in high-resolution mass spectrometry are also advancing the field by opening the door for *a posteriori* data analysis to detect compounds that were not originally targeted. The downside of GUS is that it is often labor intensive and requires extensive sample preparation. In its truest form, STA means running a prescribed panel of tests independently. In practice, toxicologists can save time and money by allowing the results of each test to help direct further testing instead of running everything independently.

1.3.3 Practical Toxicology

In an effort to use their resources efficiently, most forensic toxicology laboratories use a two-step process to detect toxicants in biological samples. The first step is screening, and is analogous to STA in that a variety of analytical procedures are employed to detect a broad range of targets. The best screening tests require little sample manipulation, are fast, inexpensive, sensitive, selective, and cover a broad range of targets. Historically, analysis using immunoassays, liquid chromatography coupled to ultra-violet detection, and gas chromatography mass spectrometry have been used for screening purposes, but liquid chromatography mass spectrometry has become a more common screening technique in recent years.[14] Screening tests may be based on multi-target screening (MTS) or may screen for a certain classification of drugs or toxins, and are generally qualitative in nature.

After qualitative identification is achieved in the screening process, the second step a toxicology laboratory will take is confirmatory analysis. This step is analogous to targeted screening because only those compounds detected in the initial screen are intentionally analyzed. While screening tests are used to detect toxins, confirmatory analysis focuses on identifying and quantifying them. According to the guidelines published by the Society of Forensic Toxicologists in conjunction with the American Academy of Forensic Science, a confirmatory test should be more specific than the screening test and based upon a different chemical principal.[15]

1.4 The Need for Something New

Traditionally, toxicology laboratories performing postmortem analyses use immunoassays, gas chromatography either alone or coupled with mass spectrometry, or liquid chromatography with ultra-violet detection to perform their screening tests. Each of these techniques suffer from various limitations including lack of specificity, difficulty of incorporating new targets, and labor intensive sample preparation and instrument maintenance.

Immunoassays are commonly used for presumptive screening in forensic toxicology because they are inexpensive, simple, fast, and automatable. Several types of

immunoassays are utilized in casework, including: fluorescence polarization immunoassays (FPIA), radioimmunoassays (RIA), enzyme-multiplied immunoassay technique (EMIT), kinetic interaction of microparticles in solution (KIMS), and enzyme-linked immunosorbent assays (ELISA).[16] Immunoassays are good at detecting a class of compounds, but have poor specificity due to the cross reactivity of the binding sites of the antibodies, which prohibits immunoassays from being used to identify specific compounds. In order for immunoassays to provide a broad range of coverage, several analyses have to be run, and it is difficult to add new targets into these analyses. Even in multiplex systems, the coverage provided by immunoassays is far from comprehensive and they have a problematic false positive rate which can waste a laboratory's time and resources by unnecessarily routing truly negative samples to confirmatory testing.

Gas chromatography, typically coupled with mass spectrometry (GC-MS) has typically been considered the so-called "gold standard" for toxicological analysis. Gas chromatography is able to separate compounds well, and when it is coupled with mass spectrometry, the technique gains a high level of specificity. GC-MS also benefits from being able to compare laboratory generated results to externally generated libraries and databases due to the reproducible ion fragmentation that is produced when using a hard ionization technique like electron impact. While GC-MS is an excellent analytical process for confirmatory testing, its use as a screening test is less than ideal because of the labor and time involved in sample preparation. Because only volatile, thermally-stable compounds can be analyzed via GC-MS, sample clean up, extraction, and derivatization is often necessary. Sample preparation in GC-MS will vary depending on the analytes of interest, making it necessary to use several different derivatizations to provide adequate coverage, making GC-MS a labor and time intensive option for postmortem toxicological screening.[14]

Liquid chromatography with ultra violet detection (LC-UV) has historically been used as a screening technique, but has recently been fading in popularity due to its limitations. LC-UV can require long run times for peak resolution and does not have good specificity. Instead of LC-UV, more toxicology laboratories are currently opting to run high performance liquid chromatography coupled with mass spectrometry (HPLC-

MS). HPLC-MS has good sensitivity and specificity, especially when high resolution or tandem mass spectrometry is used. It is also able to screen for a broad spectrum of analytes, and is not limited to volatile or thermally stable compounds like GC-MS. Because of this, HPLC-MS is rapidly gaining prominence in toxicology laboratories for confirmatory testing.[14] Its use as a screening technique, however, is less ideal for several reasons. First, HPLCs are comparatively expensive, which limits their use in public laboratories where funding is tight. This problem is compounded by the fact that HPLCs require much more oversite than most instruments in order to keep them functioning properly. When using an HPLC system, a lot of time and expertise is spent troubleshooting leaks and fluctuating backpressure, monitoring retention shifts, and preventing carryover and column degradation. The oversight and money necessary to implement HPLC-MS into routine screening testing is often beyond the capabilities of small laboratories, and even when using HPLC-MS is a viable option, the time spent in sample preparation makes HPLC-MS much better suited for confirmatory analysis rather than for initial screening.

The use of immunoassays, GC-MS, LC-UV, and HPLC-MS in conjunction with one another is an effective way for toxicology laboratories to detect a broad range of compounds commonly encountered in postmortem cases, however, they represent a significant investment of both time and money in a system that is notoriously strained by tight budgets and substantial backlogs. It would be advantageous then, if new techniques could be developed that provided a simpler, faster, and cheaper screening method that would allow toxicology laboratories to operate more efficiently.

2 INTRODUCTION

Paper spray mass spectrometry (PS-MS) is an ambient ionization technique that drastically simplifies traditional sample preparation, allowing for the rapid analysis of samples. This technique was first published in 2010 as a new method for detecting drugs and other small molecules in biofluids.[17] Originally, paper spray research was directed towards dried blood spot analysis,[18] but it has since been utilized in areas including: pharmacokinetic studies,[19] food safety,[20-23] tissue analysis,[24] and the profiling of algae and bacteria.[25,26] The ability of PS-MS to detect drugs and pharmaceuticals has been widely investigated,[27-34] routinely allowing for low to sub-ng/mL limits of quantitation.[16,18,35-38] While the focus of paper spray's application has historically been the quantitative analysis of a small panel of targets,[16] it may be well suited as an alternative for toxicological drug screening procedures as well.

2.1 Project Overview

The goal of this project was to develop a PS-MS based method to use as a screening procedure in postmortem toxicology. In order to narrow down the nearly infinite list of possible toxic substances that forensic toxicologists may encounter, the original scope for this project was defined based on consultations with the Axis Forensic Toxicology (AFT). AFT is a national toxicology lab that processes over 100 postmortem samples a day, and is accredited by the American Board of Forensic Toxicologists. AFT provided the project with a list of targets that represent their mid-grade, extended screening panel, which covers 99.5% of targets they encounter in postmortem investigations.[39] They also provided screening cutoff values for the project that generally fall at the low-end of what a therapeutic dose of the drug would be. These detection levels have since been raised at AFT, but will serve as a guide for this project. The compiled list consisted of 154 target

Figure 1 *Drug classes represented in this project. The size of each block is proportional to the number of targets in that class*

compounds (Appendix A) that represent a variety of drugs, pharmaceuticals, and metabolites. The staked column graph in Figure 1 describes the relative representation of different classes of compounds within the list of analytical targets. Classes of targets represented in the "other" category include: amphetamines, cannabinoids, cocaine, fentanyl, gastrointestinal, methadone, neurologicals, stimulants, and urologicals. The screening cutoff levels for these targets set by AFT range from 1 to 30,000 ng/mL and are summarized in Table 1.

2.2 Paper Spray-Mass Spectrometry

Using paper spray (PS) as a new approach to drug screening may allow toxicology laboratories to operate more efficiently for several reasons. First, paper spray would allow for faster data turnaround by eliminating the time spent in sample cleanup and pretreatment, and by shortening analytical run times. Being a direct analysis technique, there is no sample preparation for PS analysis, and run times are typically less than two minutes. Comparatively, the currently employed methods of GC-MS, IA's, or LC-MS have analytical run times of 10 minutes to upwards of an hour,[40-42] without considering the time spent in sample clean up and derivatization. The disposable paper substrate used in PS-MS analysis would also allow laboratories to recover the time that is currently spent troubleshooting by eliminating problems caused by carryover, clogs, and leaks.

Another reason that developing a PS-MS method for toxicological screening would be advantageous is that it provides a cheaper alternative to current techniques by

Table 1 *Screening cutoff values for the target anlaytes in the project ranged from less than 5 ng/mL to over 1000 ng/mL.*

Cutoff (ng/mL)	Number of Targets	Percentage of Total Targets
≤5	4	3%
10	17	11%
20	26	17%
25	13	8%
50	43	28%
100	14	9%
500	13	8%
1000+	24	16%

reducing cost in several different areas. First, the amount of solvent used in PS-MS analysis is typically only around 100 microliters and is completely consumed during analysis, leaving behind absolutely no solvent waste. This reduces the amount of money laboratories have to spend on reagents, as well as eliminates the costs associated with waste management and removal. Using PS-MS can also reduce the cost associated with properly storing and shipping biological samples since the dried blood spots used do not have to be carefully refrigerated like liquid whole blood or plasma samples. Another added advantage to paper spray's use of dried blood spots for sampling is that samples stored as dried blood spots are more stable than liquid samples.[43,44] The potential to lower cost, limit sample preparation, and increase sample throughput make paper spray a valuable option for toxicological screening, but there are a few things that must be considered if PS-MS is to be implemented into routine toxicology work. To understand these issues, it is important to understand the principals of paper spray-mass spectrometry.

Paper spray mass spectrometry belongs to a family of ambient ionization techniques that simplify analysis by removing the chromatography that is typically carried out prior to mass spectrometry. To accomplish this using paper spray, a sample is dried onto a piece of paper that has been cut to a point at one end. Typical sample matrices used in

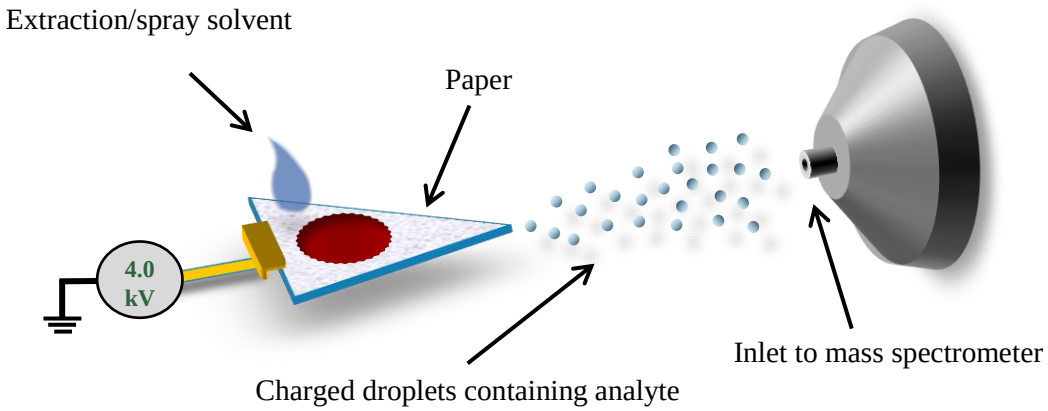


Figure 2 *Schematic of paper spray*

PS-MS analysis include whole blood, plasma, and urine. The sample is typically allowed to dry prior to analysis, although analysis of wet samples has been reported.[38] Once the sample is dried, solvent is applied to the back of the paper, which is positioned a few millimeters away from the inlet of the mass spectrometer at atmospheric pressure. As the solvent wicks through the paper and permeates the sample, it extracts the soluble components from the matrix, and they travel with the solvent front to the sharp tip of the paper. Once the paper is completely saturated, a high voltage is applied, which generates ions through electrospray ionization by inducing a Taylor cone at the sharp tip of the paper. A schematic of this experimental set up illustrated in Figure 2 is depicted visually by the images in Figure 3.

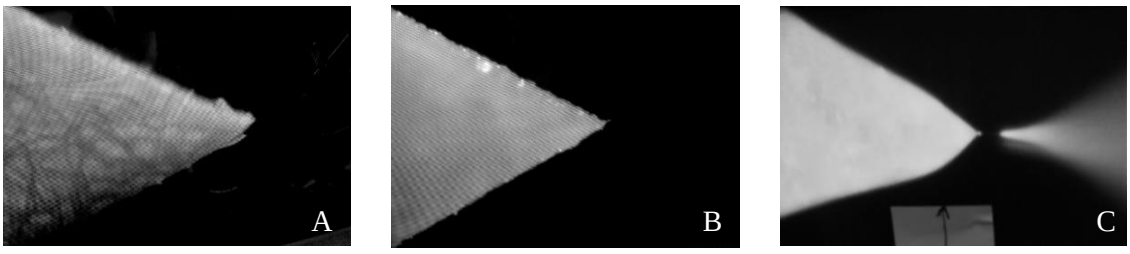


Figure 3 *(A) During paper spray, a piece of paper cut into a sharp tip is positioned 5 mm away from the inlet of a mass spectrometer. (B) Solvent is added and allowed to saturate the paper while the voltage remains off. (C) When high voltage is applied, a Taylor cone forms at the paper's tip, emitting a plume of charged droplets that quickly evaporate, leaving behind gaseous ions that enter the inlet of the mass spectrometer.*

The data that is generated though PS-MS analysis looks different than typical front-end chromatography techniques, although it is processed similarly. Because there is no intentional chromatography performed during PS-MS analysis, the term "chromatogram" is a misnomer in this application. Instead, the word "chronogram" is used to describe the ion signal over time. In typical chromatography based methods, ion signals appear as more or less Gaussian peaks. As shown in the chronogram in Figure 4, the "peaks" produced by PS-MS do not follow this trend. As soon as a high voltage is applied, the analytes extracted by the spray solvent are ionized and are detected by the mass spectrometer. As long as the paper remains wet enough to produce an electrospray, the signal will continue until the voltage is turned off. Much like typical chromatograms, chronograms are analyzed by quantifying the area under the curve of extracted ion chronograms (XIC) or selected reaction monitoring (SRM) channels.

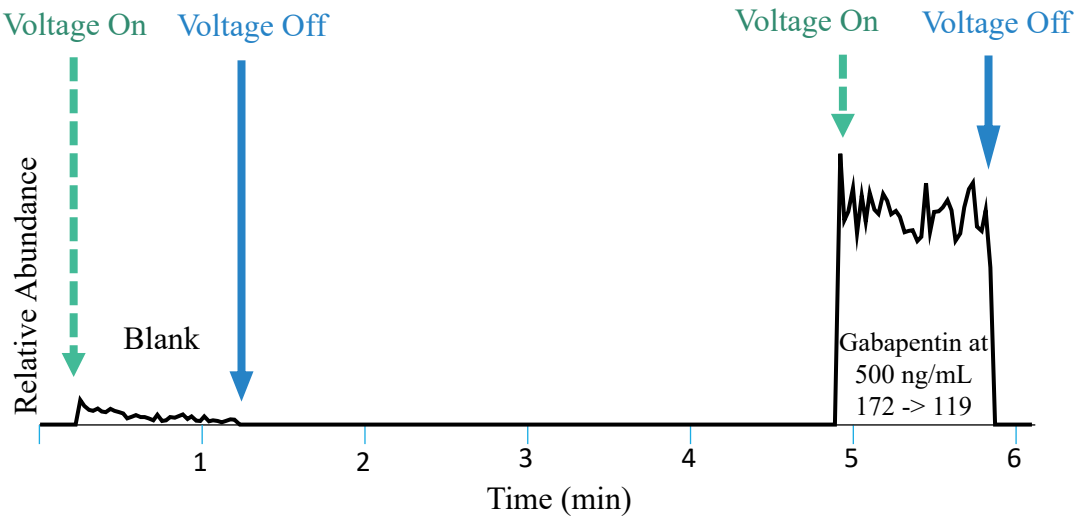


Figure 4. *Unlike typical chromatograms produced in LC-MS, the "peaks" produced in PS-MS are boxy rather than Gaussian and are called "chronograms". This is because essentially no chromatography occurs during PS-MS. When the voltage is turned on, all extracted ions enter the mass spectrometer at the same time, leading to an almost instantaneous rise in ion intensity. When the voltage is turned off, the Taylor cone collapses and ionization stops. An example chronogram of the transition from m/z 172 -> 119 for gabapentin in blank blood and at 500 ng/mL is depicted.*

2.3 Selectivity

Because no chromatography is performed in paper spray, there is a burden of specificity that is placed on the mass spectrometer. During PS-MS, all compounds that are extracted by the solvent co-elute, therefore the mass spectrometer must be able to differentiate compounds with the same nominal mass in order to discriminate between a target analyte and any of similar mass/charge that could interfere with the signal produced by the analyte of interest. In PS-MS, this is accomplished by high resolution mass spectrometry (HR-MS) and/or tandem mass spectrometry. Typical mass spectrometers that have been used for PS-MS analysis include: ion trap, orbitrap, time-of-flight, and triple quadrupole mass spectrometers.[16]

2.3.1 Mass Spectrometer Selection

While a variety of types of mass spectrometers have been used to perform PS-MS experiments, they are not all suitable for use in forensic toxicology laboratories. Ion trap mass spectrometers are able to achieve adequate specificity for PS-MS by using their MS^n feature. Because they are tandem in time, however, they are too slow to be used for screening procedures because of the need to quickly acquire data for a large list of targets. High resolution mass spectrometry is able to distinguish between co-eluting compounds based on exact mass measurements. Their high mass resolution reduces the possibility of interference from non-isomeric compounds, and they also have the unique feature of being able to retrospectively interrogate data for targets that were not originally specified. The detection limits on a HRMS are generally higher than on other mass spectrometers, however, and they are much more expensive to purchase and require daily calibration. Triple quadrupole (QqQ) mass spectrometers achieve specificity much like ion traps, in that they are able to discriminate targets by monitoring characteristic fragment ions. Triple quadrupoles are low resolution instruments and can only measure mass to about +/- 0.5 m/z units. However, because QqQ's are tandem in space rather than time, they are able to quickly scan a large list of targets by using selected reaction monitoring mode. QqQ's can also attain adequately low detection limits for toxicological

purposes and are more accessible to forensic laboratories due to their lower price. Because of these reasons, a triple quadrupole mass spectrometer was chosen as the mass spectrometer to be used during method development in this project.

2.3.2 Fragmentation

When used in selected reaction monitoring (SRM) mode, triple quadrupole instruments are able to enhance selectivity by using two sets of quadrupoles as mass filters. The first set of quadrupoles is set to only allow ions within a certain window of mass-to-charge ratio (m/z) to pass through. This window, normally 0.7 m/z units wide, filters out all other ions that have been generated by the ionization source. The ions produced by electrospray ionization and typically selected to pass through the first quadrupole are the protonated or deprotonated molecular ions ($[M + H]^+$ or $[M - H]^-$) or small adduct ions, such as potassium, sodium, or ammonium. The ions with the selected m/z, known as either parent or precursor ions, enter the second set of quadrupoles where they are bombarded with argon gas. When the ions collide with the gas molecules, they fragment via Collision Induced Dissociation (CID). The ions produced by this fragmentation process are known as daughter or product ions. The product ions then enter the third set of quadrupoles which is used to as a mass filter. In SRM mode, a characteristic fragment at a specific m/z is specified, and only that fragment is allowed to pass through the third quadrupole and reach the detector.

A decade ago, the detection of one unique fragment ion was generally seen as sufficient grounds for quantitative analysis.[45] It is more common nowadays, however, to enhance selectivity even further by monitoring more than one fragment ion per parent ion. If full specificity is required, the third quadrupole can be operated in full scan mode, in which case all of the fragments produced in the second quadrupole would be detected. For most HPLC-MS/MS applications, adequate specificity is generally achieved by monitoring two fragments for each parent ion. The most abundant of these fragment ions is referred to as the quantifier ion, while the lesser abundant is termed the qualifier ion. Typically the two most abundant ions are monitored, however, due to interfering compounds or matrix effects, other fragments can be monitored instead. Oftentimes,

however, the specificity of the chosen fragment ions is not considered due to the perceived specificity that can be gained by monitoring the ratio between different fragment ions. The idea behind using fragmentation ratios is that under specified conditions, a parent ion should fragment reproducibly, and the abundance of the fragment ions produced under those conditions should be relatively consistent from run to run for a specific target analyte.

Fragmentation ratios can be established by either taking the ratio of the intensities of the SRM transitions for each product ion or by taking the ratio of the area under the curves. There are several conditions under which fragmentation ratios may be obtained. A common method is to optimize instrumental conditions for each fragment ion separately and use the resulting ion intensities to calculate the fragmentation ratio. This method is advantageous when signal to blank response is poor or when interferences are present, as it provides an optimized signal for each product ion. Another method is to monitor two fragments under identical conditions. Other methods used include monitoring the same fragment ion under varying conditions and monitoring fragments from different precursor ions.[45] The use of the later, though, has been discouraged recently because of indications that asymmetrical signal suppression can interfere with fragmentation ratios.[46] When ionization suppression is present, competitive ionization within the matrix can hinder analytes from completely ionizing, leading to a less intense signal.

2.3.3 Selectivity in Paper Spray

By using a triple quadrupole instrument and monitoring a parent ion, two fragment ions, and the fragment ion ratio, good selectivity is achievable for paper spray even without the advantage of chromatography. Using this method should allow for differentiation between structural isomers that fragment differently. For example, although the isomers methamphetamine and 4-methylamphetamine have the same exact mass, they fragment uniquely. Methamphetamine undergoes transitions from m/z 150 to m/z 119 and 91, while 4-methylamphetamine fragments from m/z 150 to ions with m/z 133, 105, 103, 77, and 79.[16] While both compounds would produce a parent ion with m/z 150 that would pass through the first set of quadrupoles, the unique fragments generated

in the collision cell would allow one compound to be detected without interference from the other. Even in cases where no unique fragments exist, fragmentation ratios have been able to distinguish between two isomers.[45] Because of this, fragmentation ratios not only add a level of confidence to compound identification, but can also help differentiate between otherwise interfering compounds. However, in the case where isomers with the same nominal mass share the same fragment ions, unique fragments should be used if possible.

There are several established organizations that use fragments and fragmentation ratios as the basis for identification. The requirements these organizations have set for analytical identification using these ratios vary depending on application and have evolved over time. Some relevant identification criteria for LC-MS based assays are summarized in Table 2. One thing to consider when looking at these requirements is that all of these are based on the assumption that chromatography is being performed. With this, there is the assumption that co-eluting compounds will be somewhat limited, but this is not the case in paper spray. Some of these organizations acknowledge and sometimes discourage the use of commonly produced fragment ions, but none of them ban them from being used for identification purposes. Another variable to note is the tendency to base the acceptable deviation of the fragment ion ratio on the relative abundance of the two ions. The precedence for this decision is based on the European Union decision that was made by a panel of experts in the 90's.[49] Since that time, however, instrumentation has evolved and fragmentations ratios have been found to be analyte and instrument specific and vary widely based on concentration and matrix.[15,47,50,51] The variability of fragment ion ratios in some instances has caused some to suggest recently that it is better to simply rely on the presence or absence of fragment ions for compound identification.[52] Many other authors have suggested a "fitness of purpose" approach that tailors criteria to fit the analytical specificity needs of each particular project. Using fragmentation ratios in PS-MS analysis has historically been done to add a level of specificity lost by not preforming chromatography. During the method development phase of this project, setting the acceptable tolerances for these ratios will be an area of interest.

Table 2 *MS-MS based identification criteria for various applications*

Organization/Code	Applies To	Ion Requirements	Fragment Ion Ratio Tolerance
EU: 2002/657/EC[1]	Live animals and animal products	≥2 product ions	Ratio tolerance is dependent relative abundance of ion to base peak; ranges from ±20% to ±50%
EU: SANCO/12571/2013[3]	Pesticides in food products and animal feed	≥2 product ions	Ratio tolerance is ±30%
WADA TD2010DCR[5]	Sports doping	1 product if unique; if not unique, ≥2 product ions	Ratio tolerance is dependent on relative abundance of ion to base peak; ranges from ±10% to ±50%
EWDTS[6]	Workplace drug testing	≥3 ions	Ratio tolerance is ±20%
UNODC[7]	Illegal drugs	≥3 ions	Ratio tolerance is ±20%
SOFT/AAFS[8]	Forensic Toxicology	≥2 product ions	Ratio is tolerance is ±25% to ±30%

2.4 Special Considerations

There are several areas that require special consideration if paper spray mass spectrometry is to be applied to toxicological postmortem screening. Two of these are the characteristics of postmortem blood as compared to healthy blood and the matrix effects that can arise during PS-MS analysis.

2.4.1 Postmortem blood

While this project used single-donor drug free blood, there are several unique aspects of postmortem blood that will affect this project as it undergoes method validation. One important, well-recognized phenomenon that continues to be the subject of current research is the process by which drug concentrations change after death, known as post-mortem redistribution (PMR). During PMR tissue-bound drugs diffuse into adjacent blood vessels, increasing the drug concentrations in blood, especially for basic and lipophilic drugs. The extent of PMR varies with each drug, with some drugs showing highly time-dependent concentration, while other drug concentrations remain more or less stable over time.[53] Understanding the effects of PMR is one of the most important considerations for medical examiners in selecting blood sampling sites that are the truest representation of drug concentrations in the blood at the time of death. If any, the most likely effect that PRM will have on this project is an increase in basic drug concentration levels. While exact concentrations are not important for screening methods, increased concentrations could improve performance for drugs near the limit of detection when real postmortem blood is used.

Another aspect for consideration is the difference in matrix effects between ante-mortem and post-mortem blood caused by the decomposition process. Saar et al. studied the differences in matrix effects and liquid-liquid extraction (LLE) efficiency for antipsychotic drugs in ante-mortem and post-mortem blood. They found considerable differences in both extraction efficiency and matrix effects between the different types of blood and suggested that post-mortem methods be validated using drug-free postmortem blood as opposed to pooled blood bank ante-mortem blood.[54] They also found that while the matrix effects for decomposed post-mortem blood were similar to ante-mortem blood, they were much more variable. Similarly, Rosano et al found that both the variability of matrix effects and the ion suppression in post-mortem blood was higher than in bank blood.[55] Because of these differences, Peters et al. echoed Saar's proposition to use post-mortem blood in method validation and additionally suggested that non-decomposed post-mortem blood and decomposed blood be separately evaluated.[56]

In this project, higher variability in drug recovery in post-mortem blood could lead to a "hit-or-miss" scenario that could require multiple samples to be run in certain cases where the concentrations are near the limit of identification. Higher ion suppression could also be problematic for targets that either do not ionize well or exist at low concentrations. Both of these could potentially be somewhat counteracted by pre-concentration techniques, such as integrating SPE, as described by Zhang et al.[57]

2.4.2 Matrix Effects

Matrix effects are a common phenomenon which cause instrumental response to be altered due to components within the matrix in which the analyte is contained. Both the ionization technique and the analytical separation technique can affect which matrix effects are observed. For example, electrospray ionization is more affected by matrix effects than atmospheric-pressure chemical ionization.[56] In chromatography-based techniques like GC and LC, matrix effects commonly arise from co-eluting compounds and can cause either ion suppression or ion enhancement. This issue is compounded even further for non-chromatography based techniques like paper spray due to the fact that all compounds elute simultaneously.

There are two principal forms of matrix effects that affect the performance of paper spray mass spectrometry: recovery and ion suppression. Recovery refers to the percentage of the analyte that is extracted from the matrix. Ion suppression occurs when ionization efficiency for an analyte is lowered due to competitive ionization between the analyte and other co-extracted matrix components. Factors that influence competitive ionization include: access to a droplet's surface during electrospray, surface tension of the solvent, sample pH, and compound polarity.[58] Both of these affect the amount of ions that reach the mass spectrometer and are therefore available for detection. As such, they have a direct effect on the limits of detection.

Ion suppression and recovery depend on both the specific analyte of interest and the matrix from which the analyte is extracted. The spray solvent used will also play a role in ion suppression and recovery based on how soluble the analyte and potentially co-extracted matrix components are in the solvent system. Out of the typical biofluids

analyzed in PS-MS (urine, plasma, and whole blood), blood has been reported to typically have the lowest recovery. This is offset, however, by the fact that ion suppression is generally lowest in blood.[18,59] One study investigated the donor-dependency of ion suppression and recovery in blood using PS-MS and found that neither were significantly different in the 33 patient samples that were tested.[60] This suggests that within whole blood samples, the variability of matrix effects will be due mostly to compound-specific qualities.

Compounds that are known to ionize well in paper spray are hydrophobic molecules with basic aliphatic amine groups.[18] These compounds do not suffer from ion suppression nearly as much as poor ionizers.[59] Poor ionizers are hydrophilic (logP >~2) and lack basic aliphatic amine groups. Out of the 154 targets in this project, 48 do not have aliphatic amines and 97 have logP values that are greater than two. That means that 64% of the targets have the potential to ionize poorly. However, only 15% of the targets are both hydrophobic and lack a basic aliphatic amine.

The challenge to drug recovery in this project will be in maintaining the simplicity of the process. Recovery may be improved by optimizing the solvent system, but using multiple solvent systems would add time to the analytical process. However, even if multiple solvents must be run for full target coverage, the time required for PS-MS would still be competitive with current screening techniques. One of the challenges for the project, then, is to find a solvent system that efficiently extracts as many targets as possible at relevant concentrations while minimizing the possibility of extracting interfering compounds from the matrix. Ion suppression is also a potential problem, especially for poor ionizers with low target cutoff concentrations. Incorporating a pre-concentration technique with PS-MS has been shown to improve signal in some of these cases and is an option to help improve detection in future method development.[57]

While recovery and ion suppression are the primary matrix effects of concern in paper spray, other minor effects could play a role in this project, one of which is the formation of protomers. Protomers are ions that differ only by the site at which they are protonated. Where the proton is attached to the molecule is determined by the chemical environment, and solvent composition has been suggested as a contributing factor.[61] Solvent characteristics that have been found to influence the formation of protomers include: pH, aqueous-organic ratio, and ionic strength.[62]

3 MATERIALS AND METHODS

3.1 Chemicals and Reagents

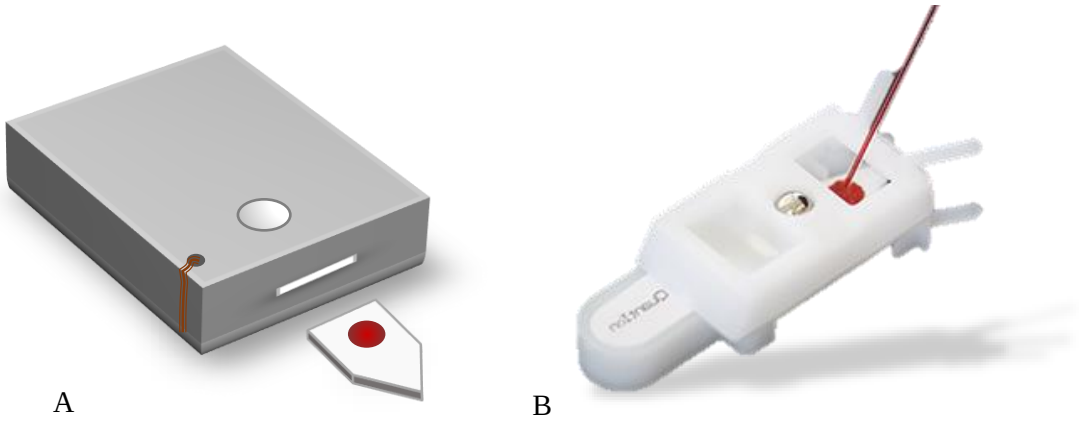
Analytical grade methanol, acetic acid, and water were purchased from Fisher Scientific (Hampton, NH, USA). All targets (Appendix A) were purchased as standards from Cerilliant (Reston, VA, USA) with the exception of: acetaminophen, metaxalone, salicylic acid, etomidate, carbamazepine, valproic acid, fluvoxamine, hydroxyzine, aripiprazole, secobarbital, amlodipine, papaverine, metoclopramide, benztropine, donepezil, ropinirole, methocarbamol, bupivacaine, levetiracetam, and labetalol, which were purchased from Sigma–Aldrich (St. Louis, MO, USA). Drug-free human blood was collected in K_2EDTA blood collection tubes from a single donor. Both analytes and blood were stored at -20°C.

3.2 Mass Spectrometer and Materials

Experiments for this project were carried out on a triple quadrupole, TSQ Vantage mass spectrometer (Thermo Scientific, San Jose, CA, USA) operated in MS/MS mode. Manually run experiments were performed using an in-house designed cartridge (Figure 5-A) and Whatman grade 31ET chromatography paper purchased from Whatman (Piscataway, NJ, USA). A TM-200 miniature CCD camera was purchased from JAI PULNiX (San Jose, CA, USA) and used to visually monitor paper and electrospray quality during manual experiments. Automated experiments were run using a Velox 360 sample handling and ionization source and Velox sample cartridges (Figure 5-B) from Prosolia, Inc. (Indianapolis, IN, USA).

3.3 Method

Two sets of methods were used during this project. Method development and initial testing were done using the manual method outlined below. After a functional method was developed using the manual method, the parameters were used to inform a method that used an automated ionization source and disposable cartridges.



A B

Figure 5: *(A) An in-house designed reusable cartridge was used for manual experiments. Using this set up 3 µL blood spots were dried onto pentagon-shaped papers hand-cut from Whatman 31ET chromatography paper and inserted into a slot at the front of the cartridge. In this design, solvent is applied through a well directly over blood spot. (B) Commercial Velox cartridges from Prosolia were used for automated experiments. In this set up, 12 µL blood spots are dried onto precut paper stored inside individual disposable cartridges. Solvent is applied into well behind blood spot and wicks through the paper and blood spot.*

For both the manual and automated experiments, analytical targets were combined together from their original stocks into 13 different cocktail solutions according to Appendix D. Each cocktail contained anywhere from 8 to 14 target analytes at 20x each analyte's target detection concentration. While the analytes were grouped according to their target detection concentrations, care was taken to ensure isomers were separated into different cocktails to avoid introducing interferences. The diluent used to bring each cocktail to the appropriate concentration was 95:5:0.01 methanol:water:formic acid, and the cocktails were stored at -20° C.

For both the manual and automated methods, 300 µL of drug-free blood was aliquoted into plastic microcentrifuge tubes and put through two freeze-thaw cycles to try to mimic the matrix decomposition that may occur in postmortem blood samples. 15 µL of one of the cocktail solutions was then added to the thawed blood so that the total organic content was less than or equal to 5%. This helped prevent the blood from congealing and limited protein precipitation which caused the blood to become heterogeneous and difficult to pipette. For blanks, 15 µL of 95:5:0.01 methanol:water:acetic acid was added to blank blood to keep organic content consistent between blank and spiked samples. The samples were then inverted 30 times, and

allowed to incubate for 45 minutes at room temperature before being aliquoted onto paper.

3.3.1 Manual Method

During manually run experiments, pentagonal papers (Figure 6-B) were razor-cut from Whatman 31ET chromatography paper to fit into an in-house designed cartridge (Figure 5-A). After the analytes were incubated in the blood for 45 minutes, the samples were mixed and 3 µL of blood was used to saturate 3.5mm paper punches (Figure 6-A) and allowed to dry at room temperature for two hours.

For analysis, the pentagonal papers were loaded though the side slot in the cartridge and then the dried blood spots were placed on top of the paper via the well on the top of the cartridge. The solvent system 95:5:0.01 methanol:water:formic acid was delivered in three 15µL aliquots to saturate the paper and then 4000 V was applied through a metal contact to induce electrospray. Data was collected for 60 s for each sample, and four replicate dried blood spots were run for each target cocktail. Between each sample, the voltage was turned off, and the cartridge was rinsed with methanol and air dried before loading the next sample. Replicate samples were run in succession followed by replicate blanks.

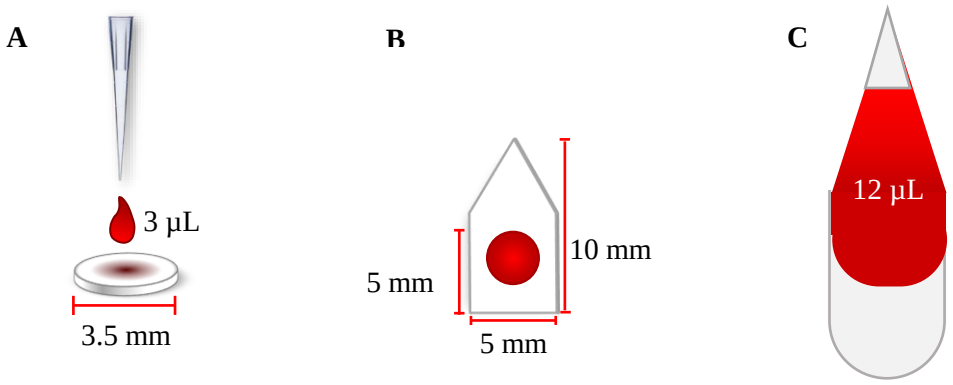


Figure 6 *(A) Using the manual method, 3 µL of whole blood was used to saturate a 3.5 mm round paper punch (B) Pentagonal paper was hand-cut with razor blades and the dried blood spot was placed on top of the paper. (C) The automated method utilized a larger, different shaped paper design than the manual method. This precut paper was housed in single-use Prosolia cartridges and 12 µL of whole blood was directly applied to the paper for analysis.*

4.3.2 Automated Method

During experiments run using the automated ionization source, blood samples were prepared following the same procedure as the manual experiments. Instead of applying the spiked blood to a paper disk and setting the disk on top of the paper tip, the spiked blood was applied directly to the paper housed inside each disposable cartridge. Two types of paper were investigated in the automated experiments, laser-cut and die-cut. Prosolia typically cuts their paper tips with lasers, but a slight brown discoloration was observed on the edges of the paper of the laser-cut tips, so die-cut tips were provided as well in order to investigate whether the laser-cutting process affected results.

Because the paper size was larger, 12 µL aliquot volumes were used in the automated experiments, and instead of a spot of blood, a band of blood was created across the width of the paper (Figure 6-C). Creating a "band" of blood instead of a spot was necessary because the automated source delivers the solvent behind the dried sample rather than on top of it. Forming a band of blood ensures that solvent must flow through the blood and will not flow around it, bypassing the sample. The larger size of the paper also led to using 120 µL of solvent rather than 45 µL, but like the manual method, the solvent was delivered in several aliquots to allow the solvent time to soak through the paper. Like the manual method, the solvent system used was 95:5:0.01 methanol:water:formic acid, and 4000 V were applied to induce electrospray. Due to the automatic ionization source, however, the spray was not able to be monitored visually for samples run in the disposable cartridges.

Unlike in the manual method, no clean up was necessary between samples while using the automatic method. Sample were allowed to dry on the papers contained within each cartridge and then the cartridges were loaded into a magazine. This magazine attached into the automated ionization source so that the samples could be queued up and left to run automatically.

4 METHOD DEVELOPMENT

Several preliminary sets of experiments were run for method development. These included a series of experiments to optimize the solvent system, paper layout, and sample loading. Experiments were also run to select appropriate SRM channels for each target analyte, establish the amount of data needed to reliably represent results, and to establish acceptable fragment ion ratio tolerances.

4.1 Solvent Selection

Solvent selection is the primary means though which both selectivity and sensitivity are manipulated during paper spray experiments. One of the challenges in this project was to find one solvent mixture that could effectively extract and spray the entire panel of analytical targets. Typically, paper spray solvents are mostly organic in composition, which allows hydrophobic small molecules like drugs to be extracted while the hydrophilic matrix components remain trapped on the paper. Examples of commonly used extraction/spray solvents include 90:10 methanol:water and 90:10 acetonitrile:water.[18] Oftentimes, acetic acid or formic acid is added as a solvent modifier at approximately 0.01% to help encourage the formation positively charged ions and to increase spray stability. In negative mode, ammonium hydroxide can be used to encourage the formation of deprotonated ions.

In order to choose a spray solvent for this project, a subset of 16 targets was chosen to represent a range of target cutoff levels, hydrophobicities, and ionizabilities. A set of two-solvent systems made by combining either methanol, acetonitrile, ethanol, isopropanol, water, dichloromethane, or tetrahydrofuran were made in proportions of 35:65, 40:60, 50:50, 65:35, and 95:5 and included 100 ppm acetic acid as a solvent modifier to encourage positive ion formation. This yielded 47 different solvent combinations that were tested on the subset of 16 targets that were spiked at their cutoff concentrations into blood. Observations for some of these solvent systems can be found in Table 3.

Table 3. *47 different solvent systems were tested for use with PS-MS in an effort to identify a solvent that would produce a steady Taylor cone at 4000 V, prevent electrical discharge, and effectively extract target analytes.*

Solvent	Observations
60:40 Ethanol:Water	Didn't produce cone
75:25 Ethanol:Water	Unstable cone, discharge at higher voltages
95:5 Ethanol:Water	Discharge that isn't solved by lowering voltage
60:40 Isopropanol:Water	Cone wasn't visualized, but spray was; wicked slowly
75:25 Isopropanol:Water	Cone wasn't visualized, but spray was; both blank and target signals elevated
95:5 Isopropanol:Water	Cone wasn't visualized, but spray was
60:40 Acetonitrile:Water	Cone wasn't visualized
75:25 Acetonitrile:Water	Cone only formed in some trials
95:5 Acetonitrile:Water	Taylor cone stable until 30s
60:40 Methanol:Water	Stable cone
75:25 Methanol:Water	Stable cone
60:40 Methanol:Acetonitrile	Cone only formed in some trials
75:25 Methanol:Acetonitrile	Produced good cone, failed after 30s
95:5 Methanol:Acetonitrile	Cone failure after 20-30 seconds; Tendency to discharge
60:40 Ethanol: Acetonitrile	Cone failure after 15 seconds, but spray still visualized for up to a minute
75:25 Ethanol: Acetonitrile	Sometimes spray was seen without a cone forming
95:5 Ethanol: Acetonitrile	Sprayed for up to a minute, even without cone formation
60:40 Isopropanol:Acetonitrile	Cone failure quickly and tendency to discharge
75:25 Isopropanol:Acetonitrile	Cone wasn't visualized, but spray was
95:5 Isopropanol:Acetonitrile	Discharges badly
40:60 Methanol:Dicholormethane	Neither cone nor spray consistently
65:35 Methanol:Dicholormethane	Unstable spray
95:5 Methanol:Dicholormethane	Cone failure after 30s and discharge
40:60 Ethanol:Dicholormethane	Neither cone nor spray consistently
65:35 Ethanol:Dicholormethane	Sputtering discharge throughout
95:5 Ethanol:Dicholormethane	Sprayed at times, but discharged
40:60 Isopropanol:Dicholormethane	Cone wasn't visualized but spray was at times
65:35 Isopropanol:Dicholormethane	Cone wasn't visualized, tendency to discharge
95:5 Isopropanol:Dicholormethane	Cone wasn't visualized but spray was
35:65 Methanol:Tetrahydrofuran	Cone wasn't visualized but spray was; Solvent depleted quickly
50:50 Methanol:Tetrahydrofuran	Cone wasn't visualized but spray was; Solvent depleted quickly
65:35 Methanol:Tetrahydrofuran	Spray dries up quickly
35:65 Ethanol:Tetrahydrofuran	Cone wasn't visualized but spray was
50:50 Ethanol:Tetrahydrofuran	Cone failure after 35s; tendency to discharge
35:65 Isopropanol:Tetrahydrofuran	Cone wasn't visualized but spray was
50:50 Isopropanol:Tetrahydrofuran	Cone wasn't visualized but spray was; tendency to discharge
65:35 Isopropanol:Tetrahydrofuran	Cone wasn't visualized but spray was; tendency to discharge

Some of these solvent systems did not produce a stable Taylor cone and had a tendency to produce an electrical discharge, which was indicated by the presence of a glowing point at the tip of the paper, a spike in the spray current, and at times an audible clicking. Other solvent systems produced unreasonably high blank signals which would not allow for adequate sensitivity. In the end, the solvent system that produced a stable electrospray and yielded the best extraction results for the panel of drugs tested was 95:5:0.01 methanol:water:acetic acid. This solvent was used for all subsequent experiments.

4.2 Paper Shape and Sample Loading

Historically, paper spay experiments have been performed on paper that is shaped more or less like an equilateral triangle on top of a square (Figure 7-A). However, during method development, elongating the paper was investigated because Vega et al. found that as the distance between the sample and the tip of the paper increased, both ionization suppression and recovery decreased.[59] This could potentially benefit targets that are bad ionizers by reducing ionization suppression, at the expense, however, of decreasing recovery. The published experiments increased the distance between the sample and the paper tip by stacking blank "spacer" discs of paper between the sample and the paper that was cut into a point. Rather than taking this approach during method development, a strip of paper 30 mm long, as depicted in Figure 7-B, was used to increase the distance from the sample to the paper tip.

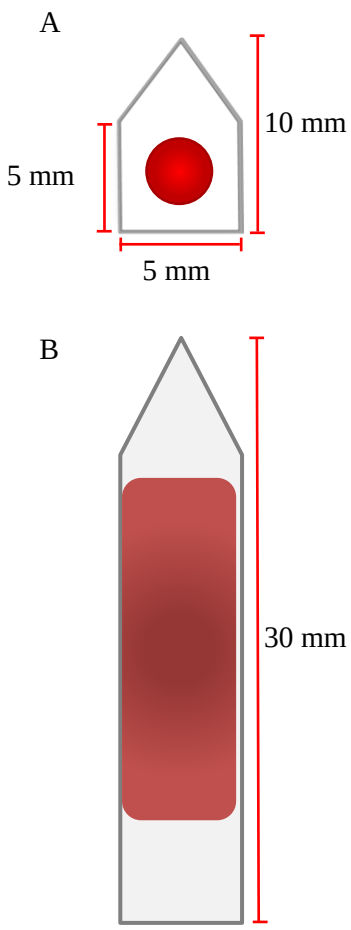


Figure 7: *Paper shape and sample loading capacity were tested on two different paper designs. (A) Traditional pentagonal-shaped papers provided a base-line reference while (B) 30 mm long pointed paper strips were used to test the effects of higher loading capacities*

4.2.1 Placement of Sample

The first set of experiments run using the long paper studied the effect of the position of the blood on the paper. To do this, 20 µL of whole blood was loaded at 13, 17, and 22 mm from the tip of the paper. All of the blood was loaded onto the paper at a single point, resulting in a sample front that was rounded due to the way that the blood wicked though the paper, but always resulting in bands of blood 11 mm wide. Five targets (morphine, zolpidem, clonazepam, fentanyl, and buprenorphine) were monitored during these experiments. The area under the curve for several fragment ions from these targets was recorded and normalized using the lowest area in order to easily compare which position produced the strongest signal. The results presented in Table 4 show that for most of the fragments, the central position at 17 mm produced the highest signal by a factor of approximately 1.5-2. During these experiments, it was observed that the cartridge design allowed some of the solvent to wick around the sides of the paper rather than through the sample, so a new cartridge was designed for future experiments. The new cartridge supported the length of the paper on small pegs rather than on channeled grooves on the sides, so that the solvent would be forced though the sample.

4.2.2 Loading Capacity and Blood Dilution

Using the small paper punches, a set of experiments were run to find the effect of diluting blood, which could improve ion suppression and/or analyte recovery. To this end, triplicate samples were run at blood:water ratios of 1:0.5, 1:1, 1:5, and 1:10. The results of the five drugs investigated (morphine, zolpidem, clonazepam, fentanyl, and buprenorphine) shown in Table 5 indicate that a dilution factor of 1:1 could increase signal to blank.

Another set of experiments were run using both diluted blood and whole blood on 30mm long paper. The longer paper allows for a larger volume of blood to be loaded onto the paper, which increases the absolute amount of drug available to be recovered during paper spray. Therefore, while using the elongated paper, the amount of sample loaded onto the paper was also investigated. The larger volumes used in these experiments meant that it was not feasible to position the samples at 17mm from the tip

as in previous experiments, since this would cause the sample to wick all the way to the tip of the paper and inhibit the solvent from spraying. Instead, the front edge of the blood spot was positioned so that it was always 6mm from the tip of the paper.

When 5µL of whole blood was used, the band of blood produced on the strip of paper was 6mm long. The same sized blood band was produced when diluted blood was used as well. The amount of blood used was increased incrementally up to 40µL, which was found to be the maximum volume of blood that the long paper strips could accommodate and maintain a good spray. The length of the blood band formed on the paper increased as the loading volume increased, with the final volume of 40µL producing a blood band 20mm long. The results of these experiments are presented in Appendix B and summarized in Figure 8 and Figure 9. The results indicate that the

Table 4 *Relative AUC of fragment ions at different positions on the long paper strip. The lowest AUC was normalized to 1.00 for comparative purposes and the position on the paper strip with the largest AUC is denoted in green.*

	Position	Relative Area Under the Curve							
m/z of fragments		**152**	**165**	**201**					
Morphine at 40 ng/mL	13 mm	1.00	1.00	1.09					
	17 mm	1.58	1.48	1.00					
	21 mm	1.40	1.33	1.02					
m/z of fragments		**235**	**92**						
Zolpidem at 10 ng/mL	13 mm	1.07	1.00						
	17 mm	1.00	1.12						
	21 mm	1.37	1.27						
m/z of fragments		**270**	**214**	**241**	**151**	**205**	**206**	**207**	**190**
Clonazepam 20 ng/mL	13 mm	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	17 mm	1.84	2.19	1.68	1.71	1.92	2.94	2.19	2.64
	21 mm	1.29	1.74	1.28	1.07	1.38	1.56	1.53	1.68
m/z of fragments		**188**	**105**	**79**					
Fentanyl at 10 ng/mL	13 mm	1.00	1.00	1.00					
	17 mm	1.07	1.53	1.79					
	21 mm	1.23	1.30	1.15					
m/z of fragments		**396**	**414**	**101**	**187**	**55**	**211**	**225**	
Buprenorphine at ng/mL	13 mm	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	17 mm	2.08	1.92	2.47	1.65	1.81	1.59	2.17	
	21 mm	1.79	1.55	1.49	1.22	1.23	1.44	1.88	

optimal loading capacity using the 30mm long paper is 20µL and that using a dilution factor of 1:1 can increase a target's signal to noise ratio by lowering the blank signal.

In order to test the effects of the optimized method of using 20µL of blood diluted 1:1 with water and placed in the center of a 30mm long paper, an experiment was run that tested this method against the original method (loading 3.5µL of whole blood onto a 3mm punch placed on top of a 10mm long paper). A panel of 13 targets was split into two groups and tested using both the original method and the optimized method. The targets from each group were cocktailed together and tested at each analyte's target cutoff concentration. Two cocktails of targets were necessary in this case to avoid introducing intra-target interferences from targets who share parent ions in the same sample, i.e. morphine and 7-aminoclonazepam.

The results of these experiments, shown in Table 6 indicate that long paper with diluted blood generally gives lower blank signals and higher response to target SRM channels, resulting in better signal to noise ratios. While in some cases, this improved S:B enough to make a target be considered "detected" due to raising the S:B ratio above 3:1, the improvements are not drastic enough to warrant adapting the entire experimental set up in favor of the simpler original method. Since the end goal of this project is to be able to run a fully automated process using the automated ionization source, the results of

Table 5 *Four dilutions schemes used to test the effect of diluting blood on the signal to blank ratio of five analytical targets. The results indicate an optimal dilution factor of 1:1*

	Dilution Factor	**1:10**	**1:5**	**1:1**	**1:0.5**
	Fragment	**S:B**	**S:B**	**S:B**	**S:B**
Morphine	286-> 152	1.7	1.7	3.5	3.0
	286-> 165	1.5	1.5	2.3	2.3
Zolpidem	308 -> 92	1.8	2.2	5.2	3.7
	308 -> 235	9.4	18	39	6.8
Clonazepam	316 -> 214	1.6	1.8	1.5	2.0
	316 -> 241	2.4	2.6	4.0	4.0
Fentanyl	337 -> 105	1.3	1.6	2.5	2.1
	337 -> 188	2.7	4.7	15	13
Buprenorphine	468-> 396	1.2	1.2	2.1	1.5
	468-> 414	1.1	1.1	2.7	1.7

the dilution and placement survey will serve to inform future work, but in this project, undiluted blood and commercially available cartridges will be tested.

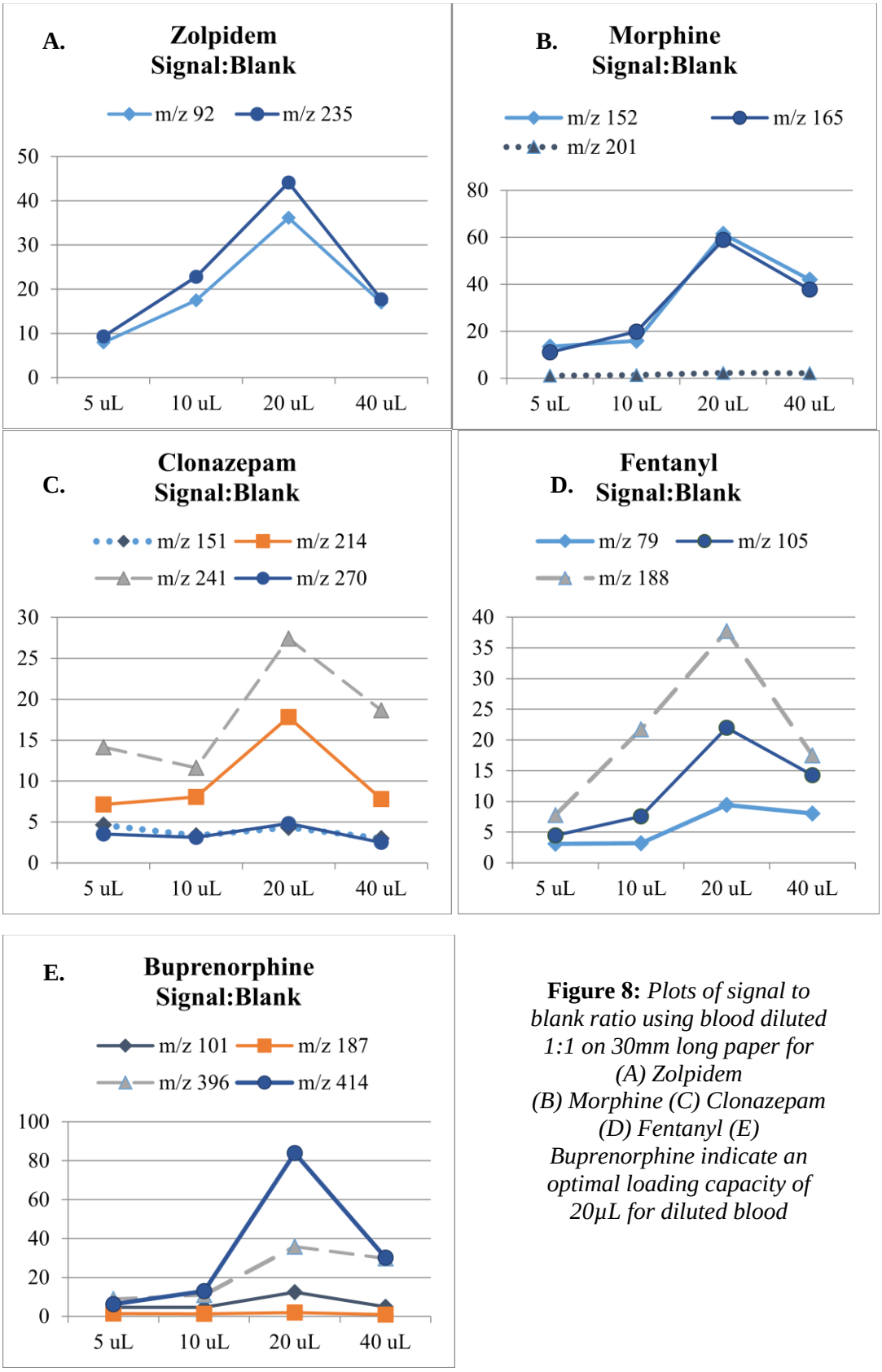


Figure 8: *Plots of signal to blank ratio using blood diluted 1:1 on 30mm long paper for (A) Zolpidem (B) Morphine (C) Clonazepam (D) Fentanyl (E) Buprenorphine indicate an optimal loading capacity of 20µL for diluted blood*

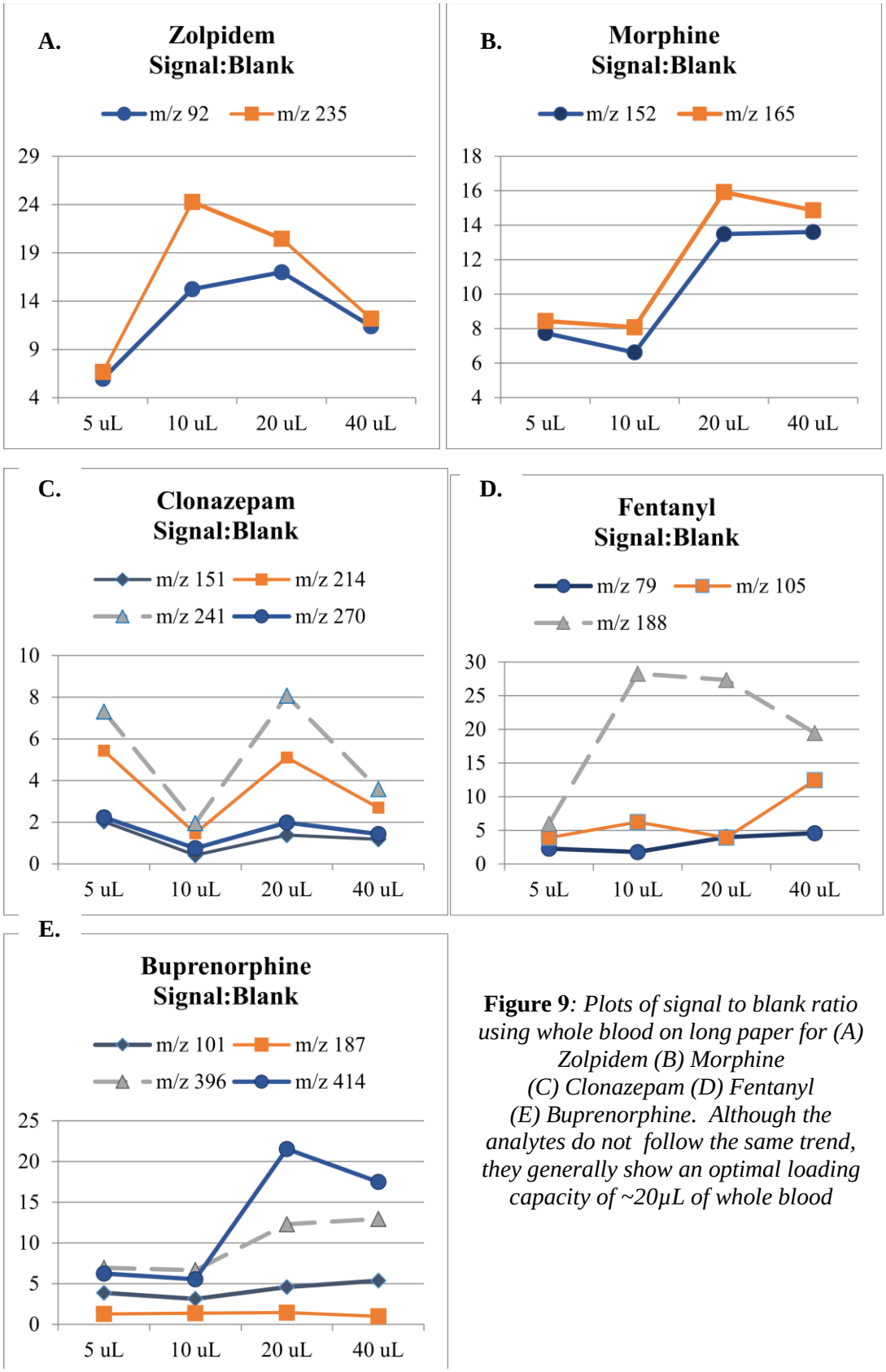


Figure 9: *Plots of signal to blank ratio using whole blood on long paper for (A) Zolpidem (B) Morphine (C) Clonazepam (D) Fentanyl (E) Buprenorphine. Although the analytes do not follow the same trend, they generally show an optimal loading capacity of ~20µL of whole blood*

Table 6 *Results from thirteen analytes show that using long paper with diluted blood generally gives lower blank signals and higher analyte response than using whole blood and 3mm punches. The first seven analytes (in blue) were run in one set of samples and the last 6 (in red) were run in a second set of samples.*

	Fragment m/z	Long Blank Avg AUC	Long Blank Error	Punch Blank Avg AUC	Punch Blank Error	Long Drug Avg AUC	Long Drug Error	Punch Drug Agv AUC	Punch Drug Error	Long S:B	Punch S:B
Gabapentin	119	1.5E+04	1400	3.2E+04	1000	2.0E+05	23000	1.6E+05	11000	**13**	**4.9**
	137	3.9E+04	3800	9.1E+04	2800	6.0E+05	69000	4.8E+05	37000	**15**	**5.2**
Normeperdine	42	2.2E+03	160	2.4E+03	290	2.2E+04	2300	1.9E+04	870	**10**	**8.1**
	56	4.4E+03	280	6.6E+03	450	4.5E+04	5300	4.2E+04	1800	**10**	**6.3**
Ketamine	125	4.1E+03	330	8.0E+03	2700	9.7E+05	1.3E+05	8.5E+05	28000	**240**	**110**
	89	3.6E+03	400	5.2E+03	1400	2.6E+05	34000	2.3E+05	8200	**72**	**44**
Meprobamate	158	7.5E+03	280	5.3E+03	620	1.3E+04	960	1.2E+04	520	**1.7**	**2.2**
	180	8.0E+03	510	3.9E+04	18000	6.6E+04	8300	9.1E+04	2400	**8.2**	**2.3**
Morphine	152	1.4E+04	1200	1.5E+04	2400	2.4E+04	3300	1.7E+04	2300	**1.7**	**1.2**
	165	5.8E+03	420	5.6E+03	1100	1.2E+04	890	8.2E+03	490	**2.0**	**1.5**
Zolpidem	92	6.6E+03	460	7.1E+03	850	9.8E+04	12000	1.1E+05	7900	**15**	**16**
	235	1.0E+03	84	4.0E+03	1540	4.9E+05	79000	5.8E+05	45000	**470**	**150**
Topiramate	207	3.3E+03	220	8.2E+03	2600	6.7E+05	1.1E+05	9.6E+05	34000	**200**	**120**
	265	8.4E+03	760	1.6E+04	5200	1.2E+06	2.1E+05	1.7E+06	58000	**150**	**100**
Alprazolam	281	8.6E+03	2200	6.3E+03	1000	5.7E+04	6900	1.5E+04	1500	**6.6**	**2.3**
	205	8.3E+03	2000	6.8E+03	860	5.1E+04	6300	1.3E+04	1700	**6.2**	**2.0**
7-Aminoclonazepam	94	9.8E+03	980	7.2E+03	800	2.0E+04	1000	9.4E+03	1100	**2.1**	**1.3**
	121	6.8E+03	980	5.7E+03	670	3.0E+04	2100	1.5E+04	1600	**4.4**	**2.7**
Buprenorphine	396	2.0E+03	340	2.3E+03	250	2.2E+03	260	1.9E+03	273	**1.1**	**0.8**
Clonazepam	214	6.0E+03	1100	5.8E+03	940	1.2E+04	1000	5.9E+03	1100	**2.0**	**1.0**
	241	3.6E+03	700	3.2E+03	500	8.7E+03	780	3.7E+03	600	**2.4**	**1.2**
Cocaethylene	196	5.3E+04	25000	1.8E+04	2600	1.9E+06	2.8E+05	1.1E+06	91000	**35**	**61**
	82	2.4E+04	8800	1.2E+04	1000	5.8E+05	82000	3.5E+05	29000	**24**	**28**
Fentanyl	105	7.3E+04	11000	7.5E+04	6900	7.9E+04	5900	8.7E+04	16000	**1.1**	**1.2**
	188	9.3E+03	860	1.4E+04	450	1.9E+04	1000	2.0E+04	830	**2.0**	**1.4**

4.3 Tuning

Thermo TSQ Tune Master was used to automatically identify the prominent fragment ions produced for each analytical target and to optimize instrumental parameters for those fragments. During tuning, the voltage was set to 4000 V, the collision gas pressure was held at 1.5 mTorr, and the software was set to exclude fragments resulting from loss of water and ammonium. From there, the software optimized instrumental conditions for the parent ion, identified the 4 most intense fragment ions, and found optimal collision energies (CE) for each fragment ion (Figure 10). For each analyte, it also optimized the S-lens, which focuses the initial gas plume that enters the inlet of the mass spectrometer into a concentrated beam of ions that enter the high-vacuum region of the mass spec.

Initial tuning was performed using targets cocktailed in 95:5:0.01 methanol:water:acetic acid at 1ppm and an H-ESI source. The results generated were the basis of selecting appropriate fragment ions and establishing fragmentation ratios and instrument parameters for each analyte. After several experiments were done, each analyte was re-tuned by spraying it in a neat solvent over blank paper at each analyte's respective target cutoff concentration. This was

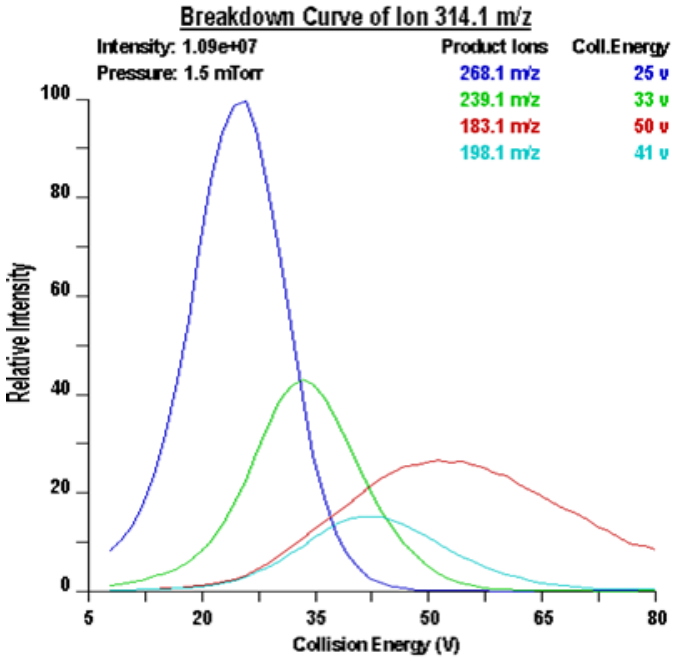


Figure 10: *An example of some of the data generated from the tuning software. In the curves produced for Flunitrazepam above, the 4 most intense fragment ions are plotted against collision energy. Some fragments, like m/z 268 are more sensitive to changes in collision energy, as is evident in the narrow peak in the curve, while other fragments with broad peaks, like m/z 183, are less effected by changes in collision energy.*

done in part because using a 1ppm solution in some cases saturated the detector, but also because of experiments performed after the initial tuning where fragmentation ratios were found to be somewhat concentration dependent. This resulted in a few minor adjustments of the fragment ions selected and the established fragment ion ratios.

In most cases, the protonated molecular ion was selected as the precursor ion, although for some targets, like topiramate, a sodiated precursor ion was used. An effort was made to encourage all targets to ionize positively due to complications that arise when paper spray is used in negative ionization mode, however, the following targets were only found at suitable intensities as deprotonated ions: all barbiturates (amobarbital, butabarbital, butalbital, pentobarbital, phentobarbital, and secobarbital), as well as furosemide, hydrochlorothiazide, warfarin, ibuprofen, salicylic acid, valproic acid, and tadalafil.

Although not typically required because of chromatography, in this project the specificity of the product ions used for identification was considered. In general, the two most abundant fragments from the automated tuning were selected, as they provide greatest sensitivity near the limit of detection. However, to enhance selectivity, larger, more complex fragments were chosen over smaller fragments when possible. Commonly produced fragments were avoided for compounds with amino side chains including m/z 58, 86, and 100.[47] Similarly, the isopropyl side chains present in many cardiovascular drugs produce common fragments at m/z 43, 60, 116, 98, 74, and 56, which were avoided if possible.[48]

In some cases, the most abundant fragment found during tuning was not used because it caused unacceptably low signal to blank ratios. This is expected in instances where the most abundant fragment is a commonly produced fragment like those outlined in Section 2.3.3. In a few instances, the most abundant fragment was not used because it caused the fragmentation ratios to behave disproportionately over a range of concentrations. While this was not observed in most cases, an example of this behavior was seen in the fragmentation of norbuprenorphine and depicted in Figure 10. Circumstances such as this have lead some to suggest that ratios should be established near the intended analytical concentration.[46] A list of the final parent/fragment ions chosen, instrumental conditions, and the fragmentation ratios used for each target analyte in this project can be found in Appendix C.

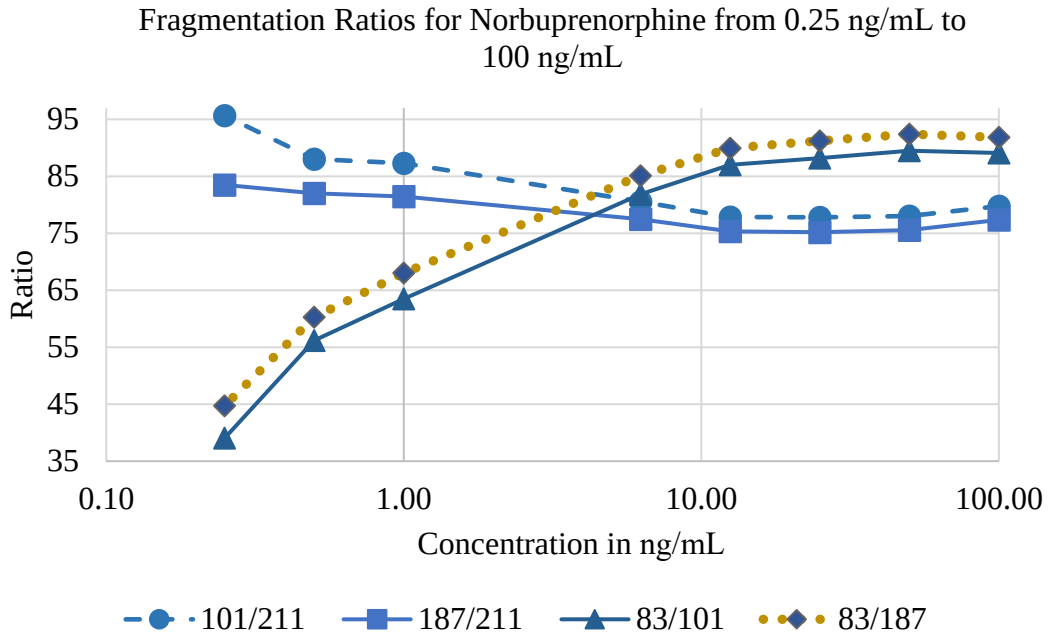


Figure 11: *When selecting appropriate fragment ions to monitor, fragmentation ratio stability over a range of concentrations was considered. For example, while m/z 83 was found to be the most abundant fragment for norbuprenorphine during tuning, ratios that use fragment m/z 83 do not behave proportionally, especially near the target detection level (1 ng/mL). In cases like this, a lesser abundant fragment is used to provide fragmentation ratios that are more consistent over a range of concentrations*

4.4 Acquisition Parameter

In tandem-in-time mass spectrometers, there are inevitable small variations in the signal intensity of fragment ions between scans. Averaging a large number of scans for each SRM channel will produce a more consistent, representative response, but at the expense of increasing run time. The variability of individual SRM channel responses becomes a compounded concern when two SRM's are used to calculate a fragmentation ratio. In order to reach an accurate ratio and to minimize the time required for sample analysis, it is necessary to determine the minimum number of scans needed in order to provide a consistent fragmentation ratio.

To this end, data from 26 different targets that had shown moderate to severe ratio deviations were analyzed. The targets were analyzed in cocktails in solution using 95:5:0.01 methonol:water:acetic acid as a solvent. The number of scans over which

data was recorded ranged from 32 to 60, and each scan lasted 0.1 seconds. The data was analyzed by monitoring the running average of the ratio of the intensities of two fragment ions per target analyte. The ratio produced after all scans were collected was considered the "true" ratio, and this ratio was compared to the ratio obtained for each target after 10, 15, 20, 25, and 30 scans. The running averages are shown for the first 50 scans in Figure 12 and Figure 13. 62% of targets showed no significant difference between their "true" fragmentation ratio and the ratio acquired after 15 scans (1.5 seconds/channel). 92% of targets showed less than ±5% variation between their true ratio and the ratio obtained after 15 scans. Doubling the number of scans to 30 only increases that percentage to 96%. As such, 15 was chosen as the minimum number of 0.1 second scans needed to provide a reproducible fragmentation ratio.

Defining the minimum number of scans needed per SRM channel has direct implications on the analysis time for paper spray. If there are 154 target analytes that each have two fragment ion channels and if each of these channels needs to be scanned for 1.5 seconds, total analysis time to screen the entire panel of targets is set at a minimum of 7.7 minutes. If run times are capped at two minutes to eliminate the need to replenish solvent mid-run, at least four samples will need to be run to provide full coverage for the purposed targets.

4.5 Screening Identification Criteria

One of the final tasks during method development for this project was to establish screening identification criteria by which the targets would be classified as "detected" or "not detected". These criteria need to be stringent enough that they support specificity and low false positive rates, but liberal enough that they allow for the detection of targets with possible interferes from co-eluting matrix components or similarly structured targets. Because paper spray cannot rely on chromatographic retention times, specificity when using a triple quadrupole instrument is obtained from identifying the presence of two known fragment ions for each target, as well as the fragmentation ratio between these ions. Confirmative results are obtained when both fragments are present in a sample and

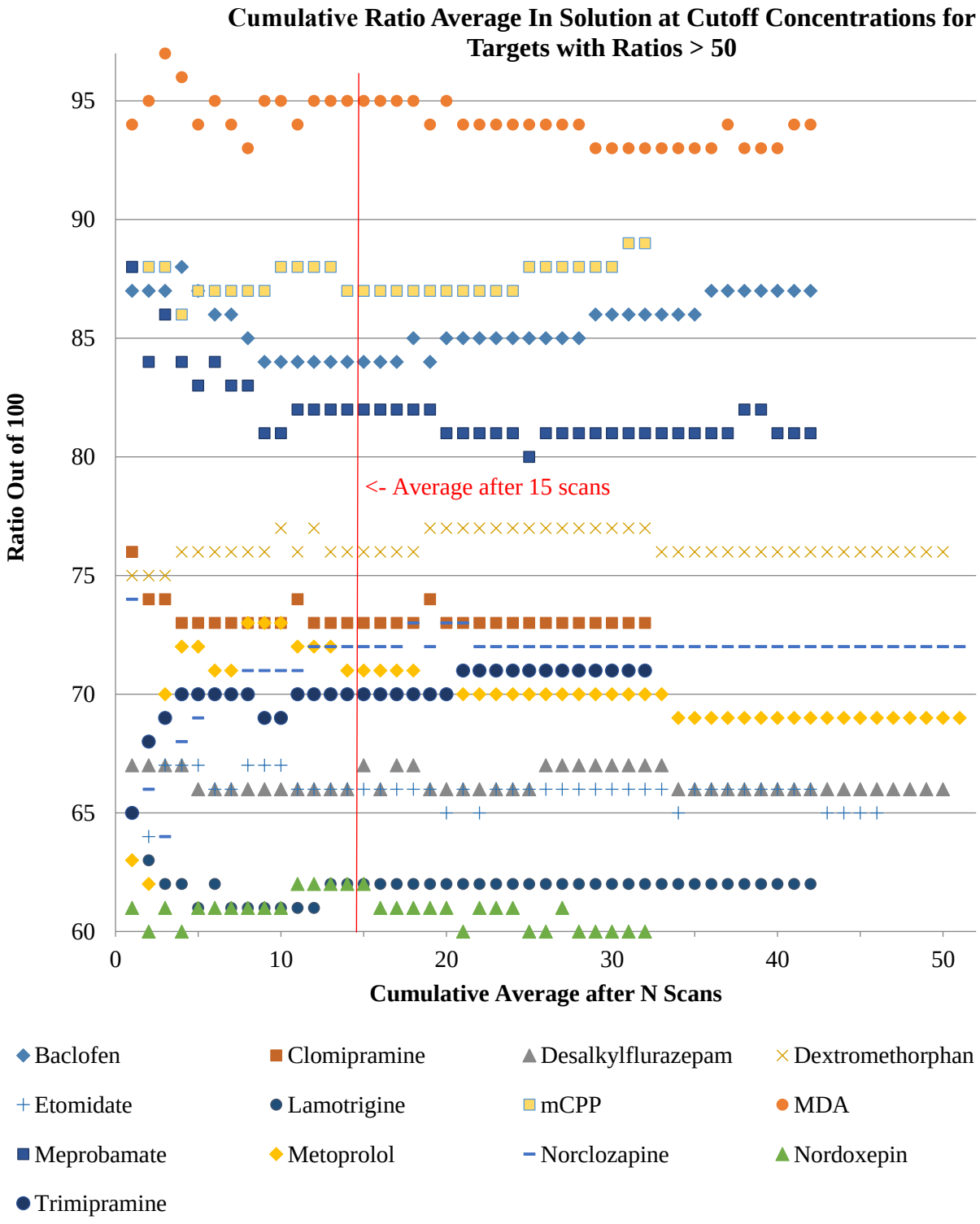


Figure 12: *Running average plot of fragmentation ratios for targets with ratios > 50 show ratios generally stabilize after 15 scans on each fragment ion channel*

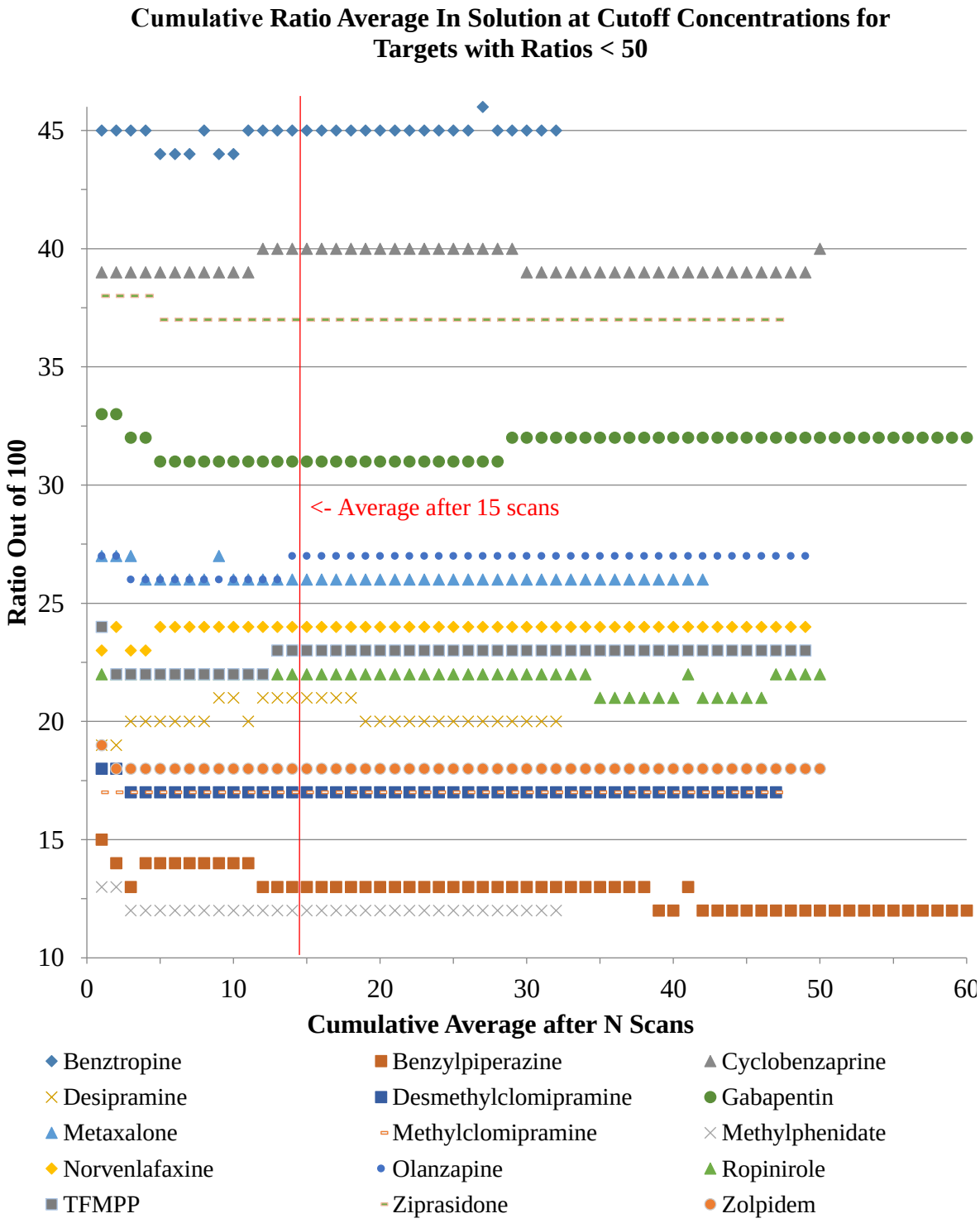


Figure 13: *Running average plot of fragmentation ratios for targets with ratios < 50 show ratios generally stabilize after 15 scans on each fragment ion channel*

when their fragmentation ratio is within some permitted tolerance of the expected ratio for the analyte. A signal to blank threshold of 3:1 is commonly used to define the limit of detection, and will be adopted in this project. Detecting two fragment ions with a signal to blank ratio of greater than 3, will be the first criteria that must be met to establish the detection of an analyte, although a higher threshold may be needed depending on future method validation experiments.

It is also necessary to establish an appropriate tolerance for fragmentation ratio variance. Establishing a fragmentation ratio tolerance that is fit for paper spray was informed by both past paper spray research and published guidelines from various organizations (Table 2). The first major guideline to be published on this topic and which many others are based off of was the European Union decision of 2002, which allowed for tolerances as high as ±50% for fragmentation ratios[3]. While these guidelines were used as a basis for establishing suitable tolerance levels for PS-MS, they are based on assumptions that do not apply to paper spray, namely the assumption that chromatography is being performed for quantitative analysis. Paper spray does not enjoy the selectivity that comes from chromatography, so the possibilities of interferences arising is higher, which suggests the need for a broader ion ratio tolerance to decrease the chances of false negatives.

A broad tolerance is also supported by the findings of Mol et al. who investigated ion ratio deviations in 120 pesticides in 21 food matrices in five different laboratories. The authors found that fragment ion ratio deviations are dependent upon detector response, and that near the limit of detection it is typical for ion ratios to diverge as much as ±45%.[49] Historically a ratio tolerance of ±20% has been used for paper spray analysis, however, qualitative screening on a large group of targets has not been done before. During quantitative analysis of eight drugs using paper spray, Espy et al. saw ratio variations as high as 19%, in the case of morphine. This variation occurred at a concentration of 38 ng/mL[34] (double this project's target LOD). Deviations at lower concentrations would be expected to be even greater because of lower detector response and would deviate even further if the ratios were derived from solvent standards and were not matrix-matched. The problem of variable ratios near LOD is illustrated by the data compiled in Figure 13. While it is possible a higher tolerance range may prove suitable

after method validation, a range of ±30% for fragment ion ratios was used in this project, as it allows more variability than previous paper spray experiments, yet still falls within the guidelines published by the society of forensic toxicologists and the academy of forensic scientists.

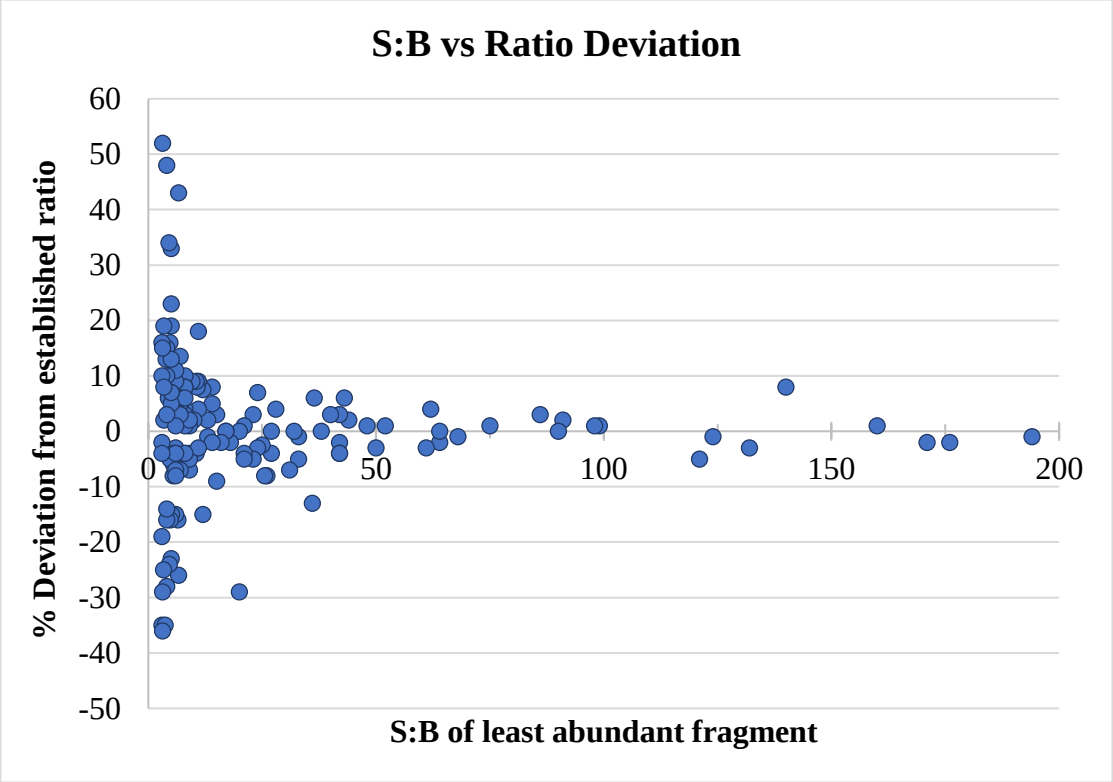


Figure 14: *Deviation between solvent-established fragmentation ratios and fragmentations ratios in whole blood at cutoff concentrations for target analytes where both fragments were detected with a S:B ≥3*

5 RESULTS

At the beginning of this project, 154 different analytical targets were proposed to investigate the feasibility of paper-spray mass spectrometry as an effective means of rapid drug screening. After method development, 14 of the originally proposed targets were shown to only ionize well in negative mode and will be studied in future experiments optimized for negative ionization. The 14 negatively ionizing targets included all of the barbiturates (amobarbital, butabarbital, butalbital, pentobarbital, phentobarbital, secobarbital), as well as: furosemide, hydrochlorothiazide, ibuprofen, salicylic acid, thiopental, tadalafil, valproic acid, and warfarin. In addition to the negatively ionizing targets, delta-9-THC and its secondary metabolite 11-nor-9-carboxy-THC, as well as buprenorphine and its metabolite norbuprenorphine were also not analyzed in these experiments because they failed to be detected in preliminary optimization experiments, even at 250 times higher than their proposed cutoff levels. Previously published paper spray studies have shown that delta-9-THC and 11-nor-9-carboxy-THC require a specialized solvent system for detection.[34] In addition to these targets, hydroxychloroquine and 10-monohydroxyoxcarbazepine have been excluded from this data set due to the availability of the compounds during the time frame in which the experiments were conducted. Excluding all of these compounds from the panel of analytical targets originally proposed by AFT left 134 targets that were analyzed to determine if PS-MS could be an effective alternative method for drug screening in forensic toxicology.

Experiments were run on paper cut manually with razors and reusable cartridges, as well as on laser-cut and die-cut paper in disposable cartridges. Only 69 of the targets, however, were run using the die-cut paper because there were only a limited number of die-cut cartridges available. Although 134 target analytes were included in the cocktails that were analyzed, results will only be reported for 133 targets using the manual method and 131 targets using the laser-cut cartridges due to an error in transposing the correct SRM channels to the mass spec acquisition program for topiramate, papaverine, and etomidate.

Out of the 134 targets included in the cocktails, 28 had to be run at concentrations

other than the cutoffs specified by AFT during project development. Six out of these 28 targets (acetaminophen, benzylpiperazine, naproxen, phenytoin, topiramate, and zonisamide) were run at concentrations lower than those originally proposed by AFT in order to avoid swamping the detector at the upper end of the calibration curve. The other 22 targets were found to have limits of detection higher than the proposed cutoff concentrations from AFT. These differences in the experimental LOD and proposed cutoffs from AFT are outlined in Table 7. Only four targets of the 23 targets whose limits of detection were found to be higher than AFT's, however, were run at concentrations outside of the expected normal therapeutic concentration range as defined by data compiled by Schultz et al. (See Table 7). The four targets were: amphetamine, buspirone, tramadol, and demoxepam.

5.1 Results

A summary of the results for all 154 proposed targets is found in Table 8 and more detailed results sorted by cocktail are found in Table 9 – Table 21. For full results, see Appendix E.

Out of the 133 targets run using the manual method, 114 met the detection requirements when spiked into drug free whole blood at the targeted cut-off concentration; the detection criteria were having a signal to blank ratio of greater than three and deviated less than 30% in their established fragmentation ratio. Nineteen of the targets failed to meet these criteria, half of the time because of low signal to blank and half of the time due to ratio variability. When the same targets (minus papaverine and etomidate whose SRM channels were wrongly transcribed) were run using the automated source and the laser-cut paper, 99 satisfied the detection criteria. In the 32 cases where the target was not detected at the cutoff or the experimental limit of detection, 15 were attributed to low signal to blank ratios and 17 to ratio variability outside of the permitted tolerance. When comparing the two methods, there were 22 instances where targets were detected manually but not when using the automated source and the laser-cut paper, and there were 7 instances where targets were detected with the automated source but not manually (See Figure 17).

Of the 68 targets (etomidate excluded due to incorrect SRM channels being monitored) that were analyzed, using the die-cut paper, 87% were detected at cutoff or LOD. Of the 8 targets that did not meet detection criteria using the die-cut paper, only two were due to low signal, while the rest were excluded due to ratio variability outside of the permitted ±30% window. There were seven instances where targets were detected using die-cut paper that were not previously detected using the automated method on laser-cut paper.

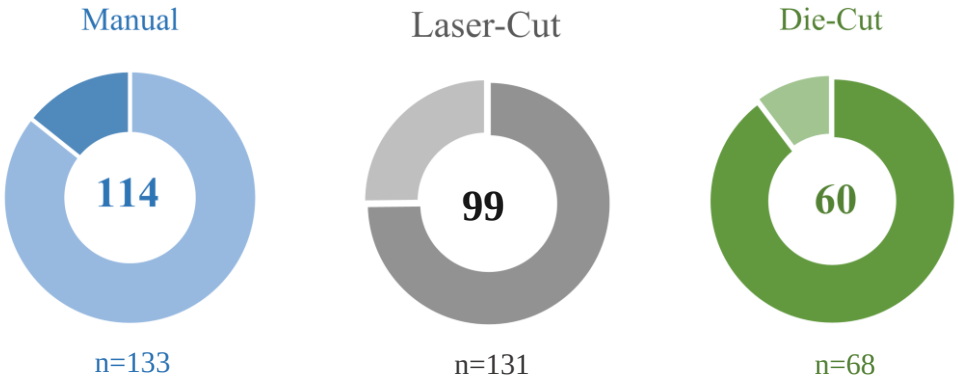


Figure 15 *Number of analytical targets detected using three different methods. Out of the 133 targets analyzed using the manual method, 86% met detection criteria; for the 131 targets analyzed using the automatic method with laser-cut paper, 76% met detection criteria. Of the 68 targets analyzed using the automatic method with die-cut paper, 87% met detection criteria*

Table 7: *A list of targets run at concentrations other than those proposed by AFT compared to normal, toxic, and fatal levels*[2] *Normal levels are defined as the effective dose where no or minimal side effects occur. Toxic levels are those at which side effects or negative symptoms arise, and fatal levels are the concentrations which result in either coma or death.*

	Concentration in mg/L				
Target	**LOD found during project**	**AFT proposed cutoff level**	**Normal**	**Toxic**	**Fatal**
6-acetylmorphine	0.03	0.02	-	-	-
7-aminoclonazepam	0.05	0.010	-	-	-
Acetaminophen	10	20	10-25	100-150	200-300
Amphetamine	0.2	0.05	0.02-0.1	0.2	0.5-1
Aripiprazole	0.1	0.05	0.15-0.5	1	-
Benzylpiperazine	0.025	0.05	-	-	-
Buspirone	0.025	0.01	0.001-0.004	.008	-
Clonazepam	0.025	0.01	0.02-0.08	0.1	-
Demoxepam	0.075	0.05	0.5-0.74	1	2.7
Desmethylclomipramine	0.1	0.02	-	-	-
Fentanyl	0.01	0.001	0.003-0.3	0.003-0.2	-
Fluvoxamine	0.025	0.02	0.060-0.23	0.5-0.65	2.8
Hydroxyzine	0.075	0.01	0.05-0.1	0.1	39
MDA	0.1	0.05	-	1.5	1.8-2
Mescaline	0.1	0.05	-	-	-
Morphine	0.025	0.02	0.01-0.1	0.1	0.1-4
Naproxen	3	30	20-50	200-400	-
Norsertraline	2	0.1	-	-	-
Nortramadol	1	0.1	-	-	-
Oxycodone	0.1	0.02	0.005-0.1	0.2	0.6
Oxymorphone	0.025	0.02	-	-	-
Paroxetine	0.025	0.02	0.01-0.05	0.35-0.4	3.7-4
Phenytoin	2.5	5	5-15	20-25	43
Topiramate	1	2	2-10	16	-
Tramadol	2	0.1	0.1-1	1	2
Ziprasidone	0.1	0.01	0.05-0.2	0.4	-
Zonisamide	7.5	10	10-40	40-70	100
Zopiclone	0.025	0.01	0.01-0.05	0.15	0.6-1.8

Table 8: *Summary of results for all 154 proposed analytical targets; "-" indicates not analyzed*

Target	Detected Manually?	Detected Laser-cut?	Detected Die-Cut?	Cocktail Run In
10-monohydroxyoxcarbazepine	Not run			-
11-nor-9-Carboxy-THC	Not run			-
6-acetylmorphine	No	Yes	Yes	G
7-aminoclonazepam	Yes	No	Yes	G
7-aminoflunitrazepam	Yes	Yes	-	J
9-hydroxyrisperidone	No	Yes	-	K
Acetaminophen	Yes	Yes	-	A
Alfentanil	Yes	Yes	Yes	G
Alpha-PVP	Yes	Yes	Yes	G
Alprazolam	No	Yes	-	K
Amitriptyline	Yes	Yes	-	L
Amlodipine	No	No	-	J
Amobarbital	Negative ionizer			-
Amphetamine	Yes	Yes	-	C
Aripiprazole	Yes	Yes	Yes	D
Atenolol	Yes	Yes	-	L
Baclofen	Yes	Yes	-	B
Benzoylecgonine	Yes	No	-	L
Benztropine	Yes	No	-	J
Benzylpiperazine	No	Yes	-	C
Brompheniramine	Yes	Yes	Yes	H
Bupivacaine	Yes	Yes	-	C
Buprenorphine	Not run			-
Bupropion	Yes	Yes	Yes	G
Buspirone	Yes	Yes	Yes	I
Butabarbital	Negative ionizer			-
Butalbital	Negative ionizer			-
Carbamazepine	Yes	Yes	-	B
Carbamazepine-10,11-epoxide	Yes	Yes	-	B
Carisoprodol	Yes	Yes	-	A
Chlordiazepoxide	Yes	Yes	Yes	G
Chlorpheniramine	Yes	Yes	Yes	I
Chlorpromazine	Yes	No	No	G
Citalopram	No	No	-	M
Clomipramine	No	Yes	-	J
Clonazepam	Yes	No	No	I
Clozapine	Yes	Yes	Yes	G
Cocaethylene	Yes	Yes	Yes	G
Cocaine	Yes	Yes	-	M
Codeine	Yes	Yes	-	M
Cyclobenzaprine	Yes	Yes	-	K
delta9-THC	Not run			-
Demoxepam	No	No	Yes	E
Desalkylflurazepam	Yes	No	No	G
Desipramine	No	No	-	J
Desmethylclomipramine	No	No	No	D
Dextromethorphan	Yes	Yes	-	K

Target	Detected Manually?	Detected Laser-cut?	Detected Die-Cut?	Cocktail Run In
Diazepam	Yes	Yes	-	L
Diltiazem	Yes	Yes	Yes	G
Diphenhydramine	Yes	Yes	Yes	H
Donepezil	Yes	Yes	Yes	F
Doxepin	Yes	Yes	-	M
Doxylamine	Yes	Yes	Yes	H
Duloxetine	No	Yes	-	J
EDDP	Yes	Yes	Yes	H
Ephedrine/ Pseudoephedrine	Yes	Yes	Yes	E
Etomidate	No	-	-	D
Felbamate	Yes	Yes	-	A
Fentanyl	Yes	Yes	-	K
Flecainide	Yes	Yes	-	C
Flunitrazepam	Yes	No	-	J
Fluoxetine	No	No	-	J
Flurazepam	Yes	Yes	Yes	H
Fluvoxamine	Yes	Yes	Yes	I
Furosemide	Negative ionizer			-
Gabapentin	Yes	No	-	C
Haloperidol	Yes	Yes	-	K
Hydrochlorothiazide	Negative ionizer			-
Hydrocodone	Yes	No	-	L
Hydromorphone	Yes	No	-	M
Hydroxychloroquine	Not Run			-
Hydroxyzine	Yes	Yes	Yes	E
Ibuprofen	Negative ionizer			-
Ketamine	Yes	Yes	Yes	D
Labetalol	Yes	Yes	Yes	F
Lamotrigine	Yes	Yes	-	B
Levetiracetam	Yes	Yes	-	A
Lidocaine	Yes	Yes	-	C
Lorazepam	Yes	Yes	Yes	H
mCPP	Yes	Yes	-	J
MDA	Yes	Yes	Yes	D
MDMA	Yes	Yes	Yes	F
MDPV	Yes	Yes	Yes	F
Meperidine	Yes	Yes	Yes	H
Mephedrone	Yes	Yes	Yes	F
Meprobamate	Yes	Yes	-	B
Mescaline	Yes	No	Yes	D
Metaxalone	Yes	Yes	-	B
Methadone	Yes	Yes	Yes	I
Methamphetamine	Yes	Yes	Yes	F
Methocarbamol	Yes	Yes	-	B
Methylone	Yes	Yes	Yes	F
Methylphenidate	Yes	Yes	-	J
Metoclopramide	Yes	Yes	Yes	D

Table 8 Continued

Target	Detected Manually?	Detected Laser-cut?	Detected Die-Cut?	Cocktail Run In
Metoprolol	Yes	No	Yes	F
Midazolam	Yes	Yes	Yes	F
Mirtazapine	Yes	Yes	Yes	F
Morphine	Yes	No	Yes	I
Naproxen	Yes	Yes	-	A
Norbuprenorphine	Not run			-
Norclozapine	Yes	No	No	F
Nordiazepam	Yes	Yes	-	M
Nordoxepin	Yes	Yes	-	J
Norfluoxetine	no	Yes	-	J
Norketamine	Yes	Yes	Yes	D
Normeperidine	Yes	Yes	Yes	H
Norpropoxyphene	Yes	Yes	Yes	E
Norsertraline	No	No	-	A
Nortramadol	Yes	Yes	-	B
Nortriptyline	Yes	Yes	-	L
Norvenlafaxine	No	No	Yes	E
Olanzapine	Yes	No	No	E
Oxazepam	No	No	-	M
Oxycodone	Yes	Yes	Yes	D
Oxymorphone	Yes	No	No	I
Papaverine	Yes	Yes	-	C
Paroxetine	Yes	Yes	Yes	I
PCP	Yes	Yes	Yes	H
Pentazocine	Yes	Yes	Yes	E
Pentobarbital	Negative ionizer			-
Phenobarbital	Negative ionizer			-
Phenytoin	Yes	Yes	-	A
Pregabalin	Yes	Yes	-	C
Primidone	Yes	No	-	A
Promethazine	Yes	Yes	Yes	H
Propoxyphene	Yes	Yes	Yes	E
Propranolol	Yes	Yes	Yes	E
Quetiapine	Yes	Yes	Yes	E
Ranitidine	Yes	Yes	-	C
Risperidone	Yes	Yes	-	K
Ropinirole	Yes	No	-	K
Salicylic Acid	Negative ionizer			-
Secobarbital	Negative ionizer			-
Sertraline	Yes	Yes	Yes	D
Sildenafil	Yes	Yes	Yes	D
Tadalafil	Negative ionizer			-
Temazepam	No	No	-	M
TFMPP	Yes	No	No	E
Thiopental	Negative ionizer			-
Topiramate	-	-	-	A
Tramadol	Yes	No	-	A
Trazodone	Yes	Yes	Yes	D
Triazolam	Yes	Yes	-	J
Trimipramine	Yes	No	-	J
Valproic Acid	Negative ionizer			-
Vardenafil	Yes	Yes	Yes	D
Venlafaxine	Yes	Yes	Yes	E
Verapamil	Yes	Yes	Yes	E
Warfarin	Negative ionizer			-
Zaleplon	Yes	No	Yes	I
Ziprasidone	No	Yes	Yes	D
Zolpidem	Yes	Yes	-	K
Zonisamide	Yes	Yes	-	B
Zopiclone	Yes	Yes	Yes	I

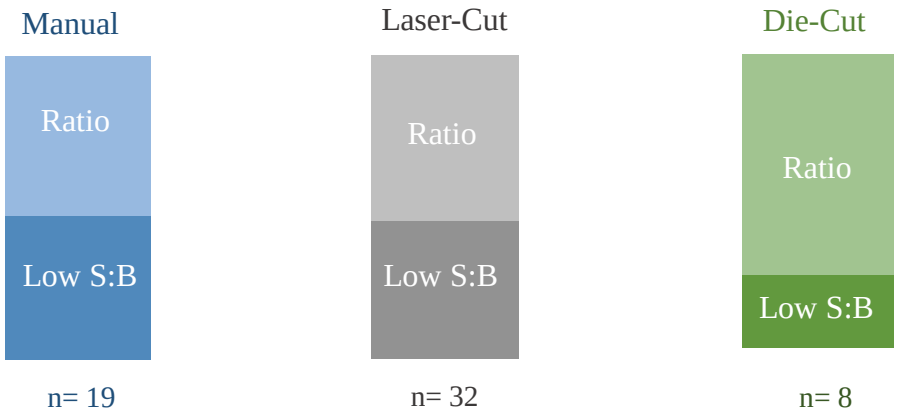


Figure 16. *Stacked column graphs representing the reason that targets failed to meet detection criteria for each of the three methods. When the manual method was used, 53% of failed detections were due to ratio deviations outside of the permitted ±30% window. For the automatic method using laser-cut paper 53% of failures were due to ratio deviations, and for die-cut experiments, the failure due to ratio deviations reached 75%.*

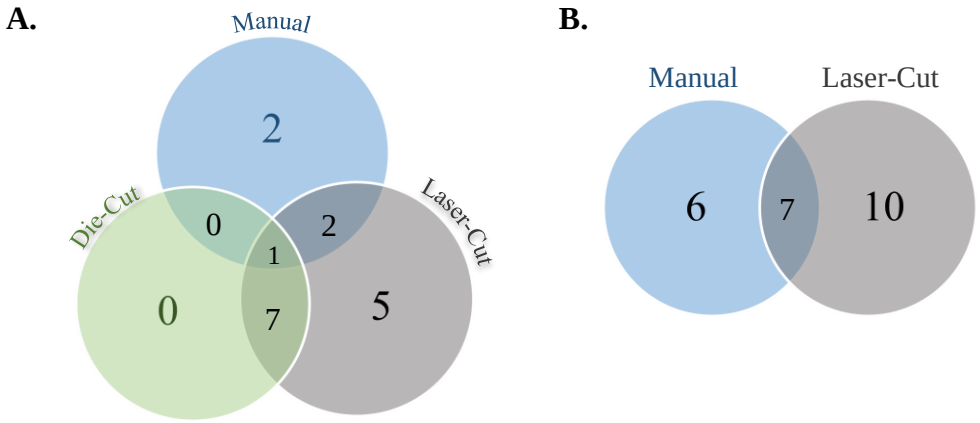


Figure 17: (*A*) *Of the 68 targets run using all three methods, 16 were detected in at least one method, but not in all three. There was one instance (desmethylclomipramine) where the target failed to be detected in any of the three methods. Venn diagram A depicts the methods where these 17 targets failed to meet detection criteria.* ***(B)*** *Of the 63 targets run using only the manual and laser-cut methods 23 total targets were not detected, 7 of which were detected in neither method. Venn diagram B specifies in which method these failures to meet detection criteria occurred.*

Table 9: *Cocktail A-Manual experiments had 26 scans per target and experiments run on the automated ionization source on the laser-cut paper had 16 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	**S:B**	Run Ratio	Ratio Deviation	True Ratio
Acetaminophen	10000	152	110	**29**	28	-5%	**139**	28	-6%	30
			65	**25**			**55**			
Levetiracetam	2000	171	126	**23**	26	6%	**48**	26	10%	24
			154	**20**			**85**			
Zonisamide	7500	213	132	**35**	48	5%	**671**	47	3%	46
			77	**20**			**31**			
Primidone	5000	219	162	**40**	72	3%	**292**	85	21%	70
			91	**15**			**2**			
Naproxen	3000	231	170	**21**	28	-9%	**7**	30	-1%	30
			185	**7**			**9**			
Felbamate	10000	239	117	**14**	61	-5%	**52**	64	-1%	64
			178	**37**			**791**			
Phenytoin	2500	253	104	**44**	66	7%	**6**	76	25%	61
			182	**43**			**12**			
Carisoprodol	2000	261	55	**4**	69	-17%	**29**	78	-6%	83
			97	**4**			**19**			
Tramadol	2000	264	264	**10**	35	17%	**2**	17 6	120%	30
			58	**39**			**5159**			
Norsertraline	2000	275	124	**35**	7	-37%	**57**	7	-36%	11
			159	**29**			**66**			
Topiramate	1000	362	265	-	-	-	-	-	-	52
			207	-			-			

Table 10: *Cocktail B-Manual experiments had 35 scans per target and experiments run on the automated ionization source on the laser-cut paper had 16 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	**S:B**	Run Ratio	Ratio Deviation	True Ratio
Baclofen	1000	214	151	**94**	84	-4%	**11**	89	2%	88
			115	**78**			**35**			
Meprobamate	2000	219	158	**208**	88	9%	**496**	87	8%	81
			97	**25**			**24**			
Metaxalone	1000	222	105	**24**	27	4%	**9**	28	10%	26
			161	**118**			**113**			
Carbamazepine	1000	237	192	**186**	22	-25%	**208**	22	-25%	30
			194	**181**			**888**			
Methocarbamol	1000	242	118	**108**	21	-10%	**109**	21	-11%	23
			199	**65**			**94**			
Nortramadol	1000	250	232	**5**	21	-17%	**5**	23	-7%	25
			42	**34**			**21**			
Carbamazepine-10,11-epoxide	1000	253	180	**246**	39	8%	**291**	38	7%	36
			210	**91**			**179**			
Lamotrigine	1000	256	145	**77**	62	0%	**83**	64	1%	63
			211	**84**			**181**			

Table 11: *Cocktail C-Manual experiments had 32 scans per target and experiments run on the automated ionization source on the laser-cut paper had 16 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Amphetamine	200	136	91	9	23	-6%	6	21	-15%	25
			119	22			47			
Pregabalin	500	160	124	68	467	0%	12	22	6%	21
			97	28			7			
Gabapentin	500	172	119	103	32	0%	34	814	2444%	32
			137	116			147			
Benzylpiperazine	25	177	85	3	21	70%	41	12	-1%	12
			91	16			34			
Lidocaine	500	235	58	311	10	-16%	59	10	-16%	12
			86	410			7086			
Bupivacaine	500	289	140	335	17	-2%	13927	17	-2%	17
			98	284			2130			
Ranitidine	500	315	102	447	51	4%	10319	51	4%	49
			176	225			713			
Papaverine	500	340	324	347	105	4%	-	-	-	101
			202	342			-			
Flecainide	500	415	301	310	40	6%	2025	40	5%	38
			98	335			5159			

Table 12: *Cocktail D- Manual experiments had 20 scans per target and experiments run on the automated ionization source had 15 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
MDA	100	180	133	**17**	93	3%	**23**	100	10%	**12**	100	10%	91
			135	**25**			**23**			**12**			
Mescaline	100	212	165	**48**	64	-20%	**55**	62	-23%	**7**	30	-63%	81
			180	**6**			**4**			**2**			
Aripiprazole	100	448	176	**35**	29	1%	**45**	28	2%	**56**	28	1%	28
			285	**74**			**391**			**215**			
Desmethylclomipramine	100	301	227	**11**	24	39%	**30**	26	51%	**13**	23	37%	17
			72	**108**			**1720**			**1232**			
Etomidate	100	245	141	**20**	105	41%	-	-	-	-	-	-	66
			95	**5**			**29**			**33**			
Ketamine	100	238	125	**141**	25	1%	**1544**	25	3%	**157**	26	6%	24
			89	**91**			**209**			**31**			
Metoclopramide	100	300	184	**89**	37	-4%	**1451**	39	1%	**249**	38	-3%	39
			227	**110**			**6443**			**1591**			
Norketamine	100	224	125	**108**	45	2%	**543**	45	2%	**118**	46	5%	44
			179	**67**			**159**			**32**			
Oxycodone	100	316	212	**19**	65	4%	**25**	68	7%	**10**	69	10%	63
			241	**29**			**61**			**33**			
Sertraline	100	306	159	**31**	81	-4%	**72**	84	-1%	**47**	82	-3%	85
			275	**81**			**351**			**349**			

Table 12 *Continued*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Sildenafil	100	475	100	**143**	69	12%	**469**	66	6%	**499**	68	9%	62
			283	**25**			**47**			**20**			
Trazodone	100	372	148	**76**	83	-1%	**914**	83	-2%	**167**	83	-2%	84
			176	**71**			**316**			**433**			
Vardenafil	100	489	151	**39**	40	-9%	**268**	42	-2%	**207**	41	-5%	43
			312	**99**			**474**			**235**			
Ziprasidone	100	413	159	**6**	81	121%	**8**	44	19%	**6**	45	22%	37
			194	**129**			**125**			**88**			

Table 13: *Cocktail E- Manual experiments had 20 scans per target and experiments run on the automated ionization source had 15 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	**S:B**	Run Ratio	Ratio Deviation	**S:B**	Run Ratio	Ratio Deviation	True Ratio
Ephedrine/Pseudoephedrine	50	166	115	**26**	83	-3%	**44**	83	-4%	**59**	85	-2%	86
			117	**27**			**50**			**21**			
TFMPP	50	231	118	**22**	24	4%	**20**	79	242%	**4**	8	-64%	23
			188	**42**			**35**			**76**			
Propranolol	50	260	116	**44**	89	1%	**558**	91	3%	**88**	91	3%	88
			183	**23**			**72**			**33**			
Norvenlafaxine	50	264	107	**13**	32	34%	**6**	26	9%	**2**	34	40%	24
			58	**17**			**487**			**40**			
Venlafaxine	50	278	121	**20**	26	4%	**16**	26	5%	**5**	32	26%	25
			58	**93**			**894**			**171**			
Pentazocine	50	286	218	**72**	24	8%	**774**	22	2%	**186**	24	8%	22
			69	**11**			**17**			**11**			
Demoxepam	75	287	180	**6**	79	27%	**18**	67	2%	**4**	70	8%	65
			207	**3**			**10**			**3**			
Norpropoxyphene	50	308	128	**20**	49	3%	**71**	49	2%	**17**	51	6%	48
			44	**61**			**852**			**416**			
Olanzapine	50	313	198	**11**	27	3%	**2**	33	22%	**5**	40	48%	27
			256	**28**			**39**			**32**			

Table 13 continued

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	**S:B**	Run Ratio	Ratio Deviation	**S:B**	Run Ratio	Ratio Deviation	True Ratio
Propoxyphene	50	340	128	**10**	21	3%	**22**	20	-1%	**10**	21	6%	20
			266	**31**			**425**			**227**			
Hydroxyzine	75	375	165	**56**	83	-3%	**1903**	84	-2%	**702**	84	-2%	86
			201	**28**			**124**			**135**			
Quetiapine	50	384	221	**81**	64	0%	**812**	65	1%	**284**	65	1%	64
			253	**66**			**1080**			**648**			
Verapamil	50	455	150	**80**	37	2%	**1463**	37	3%	**241**	37	3%	36
			165	**34**			**647**			**335**			

Table 14: *Cocktail F-Manual experiments had 27 scans per target and experiments run on the automated ionization source had 20 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Methamphetamine	50	150	119	**98**	38	-4%	**278**	38	-2%	**330**	38	-2%	39
			91	**51**			**69**			**105**			
Mephedrone	50	178	144	**112**	75	-2%	**279**	75	-1%	**114**	75	-1%	76
			145	**78**			**230**			**132**			
MDMA	50	194	105	**40**	36	5%	**23**	37	5%	**20**	38	9%	35
			163	**114**			**676**			**392**			
Methylone	50	208	132	**36**	53	1%	**45**	53	1%	**16**	54	4%	52
			160	**58**			**216**			**31**			
Mirtazapine	50	266	194	**101**	40	-1%	**52**	41	-1%	**26**	42	2%	41
			195	**109**			**329**			**168**			
Metoprolol	50	268	116	**70**	75	10%	**985**	72	6%	**365**	89	31%	68
			77	**11**			**18**			**6**			
MDPV	50	276	135	**84**	89	0%	**90**	89	0%	**31**	94	6%	89
			175	**92**			**309**			**162**			
Norclozapine	50	313	192	**68**	74	2%	**181**	137	87%	**90**	135	85%	73
			270	**59**			**84**			**45**			
Midazolam	50	326	249	**44**	28	3%	**1487**	28	3%	**337**	28	4%	27
			291	**45**			**11116**			**3530**			
Labetalol	50	329	162	**41**	95	10%	**51**	107	23%	**24**	105	21%	87
			91	**81**			**351**			**349**			
Donepezil	50	380	243	**62**	16	-2%	**701**	16	0%	**248**	16	2%	16
			91	**75**			**622**			**272**			

Table 15: *Cocktail G-Manual experiments had 24 scans per target and experiments run on the automated ionization source had 20 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Alpha-PVP	50	232	91	54	41	1%	423	41	0%	126	42	3%	41
			77	24			49			10			
Bupropion	50	240	184	47	56	1%	155	56	2%	35	57	3%	55
			131	49			198			46			
7-aminoclonazepam	50	286	121	19	39	9%	12	44	21%	2	68	88%	36
			94	9			5			1			
Desalkylflurazepam	50	289	140	6	48	-27%	13	150	127%	8	241	266%	66
			226	44			96			11			
Chlordiazepoxide	50	300	227	26	24	5%	59	25	14%	19	27	21%	22
			255	18			10			4			
Cocaethylene	50	318	196	62	27	-1%	799	26	-3%	350	27	-1%	27
			82	42			846			127			
Chlorpromazine	50	319	214	19	30	18%	97	385	1380%	11	365	1305%	26
			58	22			107			50			
Clozapine	50	327	270	77	64	0%	1634	65	1%	306	64	0%	64
			192	64			864			214			
6-acetylmorphine	30	328	165	2	42	-26%	6	52	-9%	7	59	4%	57
			211	2			6			4			
Diltiazem	50	415	178	76	43	-1%	8926	44	-1%	823	43	-1%	44
			150	56			2166			263			
Alfentanil	50	417	268	27	68	10%	458	62	1%	318	67	7%	62
			197	13			622			272			

Table 16: *Cocktail H Manual experiments had 28 scans per target and experiments run on the automated ionization source had 21 scans per target.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Normeperidine	25	234	160	11	23	1%	39	22	-2%	7	20	-7%	22
			56	9			81			14			
PCP	25	244	91	9	55	-3%	28	51	-9%	19	48	-15%	56
			159	8			93			60			
Meperidine	25	248	220	16	57	-1%	253	60	3%	29	62	6%	58
			174	12			99			14			
Diphenhydramine	25	256	167	12	44	3%	200	44	2%	110	45	4%	43
			165	16			230			93			
Doxylamine	25	271	167	16	88	0%	126	85	-3%	67	88	0%	88
			182	18			307			106			
EDDP	25	278	234	12	41	-1%	1410	41	-2%	491	41	-3%	42
			249	13			2742			619			
Promethazine	25	285	86	6	43	-1%	17	44	2%	17	45	4%	43
			198	6			16			12			
Brompheniramine	25	319	274	21	50	5%	719	48	2%	200	49	4%	47
			167	9			248			98			
Lorazepam	25	321	276	22	49	2%	295	47	-1%	159	47	-1%	48
			167	11			268			111			
Flurazepam	25	388	315	17	23	5%	1975	23	3%	761	23	4%	22
			317	12			572			48			

Table 17: *Cocktail I-Manual experiments had 28 scans per target and experiments run on the automated ionization source had 21 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Diecut**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Chlorpheniramine	25	275	230	43	51	-4%	1192	50	-5%	26	50	-5%	53
			167	28			1324			96			
Morphine	25	286	152	4	79	12%	4	63	-12%	2	62	-12%	71
			165	3			5			2			
Oxymorphone	25	302	227	8	77	6%	12	100	37%	3	112	53%	73
			198	8			7			1			
Zaleplon	25	306	236	16	88	0%	18	99	13%	1	68	-23%	88
			264	15			8			3			
Methadone	25	310	265	36	33	1%	1763	33	3%	251	32	1%	32
			105	23			306			59			
Clonazepam	25	316	270	5	29	-8%	3	24	-26%	1	44	39%	32
			214	10			4			1			
Fluvoxamine	25	319	71	10	25	16%	37	24	13%	11	23	10%	21
			200	4			13			4			
Paroxetine	25	330	192	9	59	13%	26	57	9%	8	51	-3%	52
			70	6			11			8			
Buspirone	25	386	122	36	23	28%	1977	18	1%	118	19	3%	18
			95	5			91			15			
Zopiclone	25	389	245	5	35	-11%	7	37	-6%	4	33	-15%	39
			112	18			7			11			

Table 18: *Cocktail J-Manual experiments had 19 scans per target and experiments run on the automated ionization source had 18 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
mCPP	20	197	154	6	81	-9%	8	90	1%	89
			118	13			4			
Methylphenidate	20	234	84	12	13	5%	114	13	4%	13
			56	6			5			
Nordoxepin	20	266	107	8	59	-1%	10	56	-7%	60
			235	14			36			
Desipramine	20	267	72	17	37	79%	249	27	34%	20
			193	6			5			
7-aminoflunitrazepam	20	284	135	11	51	0%	9	55	8%	51
			227	13			8			
Trimipramine	20	295	193	8	78	10%	25	100	41%	71
			208	8			26			
Norfluoxetine	20	296	134	3	19	-25%	4	19	-28%	26
			30	10			76			
Duloxetine	20	298	44	2	95	10%	14	88	1%	87
			154	2			3			
Benztropine	10	308	167	13	45	-1%	195	1	-98%	45
			165	13			14			
Fluoxetine	20	310	44	15	16	30%	219	17	34%	13
			148	6			3			
Flunitrazepam	20	314	268	3	32	-16%	2	23	-40%	38
			239	7			5			
Clomipramine	20	315	227	4	49	-33%	10	67	-8%	73
			242	5			16			
Triazolam	20	343	308	13	85	11%	336	80	6%	76
			239	5			13			
Amlodipine	20	409	238	2	77	14%	17	79	18%	67
			294	2			2			

Table 19: *Cocktail K-Manual experiments had 30 scans per target and experiments run on the automated ionization source had 18 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Ropinirole	10	261	114	5	22	3%	8	90	327%	21
			132	4			4			
Dextromethorphan	10	272	215	5	77	1%	23	81	7%	76
			147	5			11			
Cyclobenzaprine	10	276	215	5	39	-1%	150	42	7%	39
			231	5			23			
Zolpidem	10	308	92	4	19	5%	25	20	11%	18
			235	5			23			
Alprazolam	5	309	205	3	87	0%	6	81	-7%	87
			281	3			4			
Fentanyl	10	337	188	5	76	9%	13	75	7%	70
			105	4			26			
Haloperidol	10	376	123	4	93	0%	39	91	-3%	93
			165	4			40			
Risperidone	10	411	191	3	8	3%	141	8	4%	8
			110	3			19			
9-hydroxyrisperidone	10	427	207	3	22	1%	79	24	9%	22
			110	3			16			

Table 20: *Cocktail L-Manual experiments had 14 scans per target and experiments run on the automated ionization source had 19 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Nortriptyline	20	264	233	31	45	6%	55	44	4%	42
			117	12			23			
Atenolol	100	267	145	6	70	-10%	45	77	-1%	78
			190	12			151			
Amitriptyline	20	278	233	21	62	1%	37	59	-4%	61
			117	14			46			
Diazepam	50	285	154	18	78	-3%	19	81	0%	81
			222	22			34			
Benzoylecgonine	50	290	168	11	38	16%	77	51	55%	33
			77	4			4			
Hydrocodone	20	300	199	19	40	10%	17	56	54%	37
			171	10			5			

Table 21: *Cocktail M- Manual experiments had 13 scans per target and experiments run on the automated ionization source had 18 scans per target. Red shading indicates failure to meet detection criteria.*

	Concentration in blood (ng/mL)	*Precursor m/z*	*Product m/z*	**Manual**			**Lasercut**			
				S:B	Run Ratio	Ratio Deviation	S:B	Run Ratio	Ratio Deviation	True Ratio
Nordiazepam	50	271	140	44	72	6%	25	72	-6%	68
			208	29			16			
Doxepin	20	280	107	12	48	-8%	28	51	-2%	52
			165	15			28			
Hydromorphone	20	286	185	6	77	11%	3	105	53%	69
			157	4			3			
Oxazepam	50	287	241	2	89	511%	2	188	1151%	15
			77	1			2			
Codeine	20	300	215	10	74	23%	4	46	-25%	61
			199	4			5			
Temazepam	50	301	177	2	26	-17%	7	47	48%	32
			239	3			3			
Cocaine	50	304	182	46	23	8%	97	22	2%	21
			82	14			49			
Citalopram	10	325	262	16	78	66%	28	68	44%	47
			234	6			12			

6 INTERFERENCE STUDY

In order for paper spray to be a viable approach to post-mortem toxicological screening it must be able to differentiate target analytes from endogenous substances that exist within a matrix, and it must also be able to differentiate target analytes from other exogenous compounds. For example, PCP is one of the analytical targets of the developed screening method and is categorized as a schedule II controlled substance in the US, but it shares the same molecular weight as frovatriptan, a prescription migraine medication. In this instance, the legal medication could affect the results for a controlled substance by producing a parent ion with the same mass to charge ratio. It is therefore necessary to investigate the specificity of the developed method in producing accurate results in the presence of potentially interfering compounds.

Interferences to the target analytes can arise from several different situations. Both exogenous and endogenous isomers of an analyte may produce common daughter fragments with the target analyte, which would result in skewed fragmentation ratios and could create false negative results. Compounds that do not share the same mass as the target could even interfere by producing adduct ions more complex than $[M+H]^+$ that have the same mass to charge ratio as the protonated adduct of the target analyte. Isotopic interferences could also arise when molecules with moieties containing heavily abundant isotopes, such as chlorine or bromine, are present. To a lesser extent, isotopic interferences in larger molecules could even arise due to the C-13 isotope if the interfering compound is found at a much higher concentration than the target analyte. Although this makes the list of potential interferences for the project's 154 different analytical targets practically unquantifiable, investigating, to a reasonable extent, potentially interfering compounds will help inform data interpretation.

To investigate compounds that could cause interferences with the developed drug screening method, potential interferences were sorted into two classifications: interferences that arise from exogenous substances potentially contained within blood, and intra-target interferences that arise from isomeric or isotopic parent ion overlap between the 154 analytical targets defined in the study.

6.1 Exogenous interferences

While there are compounds that could produce isotopic interferences by generating the same parent ions as the target analytes in this project, these compounds would have to contain elements with relatively abundant isotopes (i.e. chlorine, bromine) and would have to be fairly concentrated within the matrix to become a true concern. As the scope of identifying potentially interfering compounds is already wide, at this time the search has been limited to compounds that are biologically available to the matrix and have the same molecular weight as the target analytes. To this end, two publically available databases were used to compile a list of potentially interfering compounds: the Human Metabolome Database[63-65], a database of small molecule metabolites, and DrugBank, a searchable database that contains over 8232 drugs.[66] Using these databases, 450 potentially interfering exogenous compounds were identified that have the same molecular weight at the target analytes of this project. These compounds are outlined in Appendix F.

6.2 Intra-target interferences

Out of the 154 analytical targets in this study, 43 positively ionizing analytes were identified that that have parent ions that share the same mass to charge ratio as another analyte, and could therefore be a source of interference. These target analytes are presented in Table 22. For an analyte to be a true interference to another target, it must produce at least one fragment as another target with the same parent ion, and it must produce this fragment in an abundance such that it alters the expected fragmentation ratio of the target analyte. To this end, each of the 43 potentially interfering targets were spiked at 1ppm into 95:5:0.01 methanol:water:acetic acid and introduced into the mass spec via an ESI ionization source, where they were scanned for 60s for the daughter fragments of all the potentially interfering target analytes. Each infusion was followed by a 60 second infusion of blank solvent to avoid carryover. Topiramate was added to each solution at 100 ng/mL as an internal standard to monitor ionization. The signal-to-blank for topiramate never fell below 15:1 during all of the interference experiments, and the fragmentation ratio never deviated more than 8% from the established ratio.

During these preliminary experiments, 25 target analytes were identified that had a signal-to-blank greater than 3:1 when monitoring the SRM channels for a different analyte. The results for the initial experiments using the ESI spray source are presented in Table 23. These targets were investigated further, while the other 18 analytes were concluded to not be true interferences since they added no significant increase in signal at 1000 ng/mL, a concentration much higher than the cutoff levels for most of the targets.

Table 22: *Targets that share the same parent ion and could potentially interfere with one another.*

Name	Cutoff in ng/mL	MW	Parent Ion	Product Ion 1	Product Ion 2	Ion Ratio
Primidone	5000	218	219.087	162.111	91.055	100:70
Meprobamate	2000	218	219.096	158.134	97.105	100:101
Amobarbital	500	226	225.116	182.139	42.066	100:47
Pentobarbital	500	226	225.116	182.144	42.043	100:45
Normeperidine	25	233	234.121	160.129	56.068	100:22
Methylphenidate	20	233	234.122	84.086	56.072	100:12
Phenytoin	5000	252	253.081	182.110	104.046	100:65
Carbamazepine-10,11-epoxide	1000	252	253.088	180.092	210.110	100:36
Lamotrigine	1000	255	256.010	210.993	144.949	100:65
Diphenhydramine	25	255	256.129	167.098	165.073	100:48
Carisoprodol	2000	260	261.144	97.097	55.065	100:81
Ropinirole	10	260	261.145	114.128	86.099	100:25
Nordoxepin	20	265	266.120	107.042	235.118	100:57
Mirtazapine	50	265	266.133	195.104	194.091	100:41
Norsertraline	100	291	275.030	158.974	123.990	100:26
Chlorpheniramine	25	274	275.104	230.082	167.072	100:54

Table 22 Continued

Name	Cutoff in ng/mL	MW	Parent Ion	Product Ion 1	Product Ion 2	Ion Ratio
MDPV	50	275	276.127	175.086	135.041	100:91
Cyclobenzaprine	10	275	276.141	215.092	216.106	100:56
EDDP	25	277	278.146	234.128	249.166	100:51
Venlafaxine	50	277	278.154	58.083	121.063	100:26
7-aminoclonazepam	10	285	286.057	222.134	121.109	100:85
Morphine	20	285	286.107	152.057	165.062	100:70
Pentazocine	50	285	286.162	218.155	69.068	100:29
Desalkylflurazepam	50	288	289.035	140.015	226.092	100:66
Bupivacaine	500	288	289.165	140.126	98.094	100:19
Chlordiazepoxide	50	299	300.070	227.044	241.056	100:27
Metoclopramide	100	299	300.125	227.057	184.026	100:51
Sertraline	100	305	306.067	158.971	275.068	100:84
Zaleplon	25	305	306.106	236.088	264.123	100:85
Zolpidem	10	307	308.136	235.158	92.076	100:19
Benztropine	10	307	308.149	167.074	165.069	100:68
Norpropoxyphene	50	325	308.155	100.064	44.064	100:71
Fluoxetine	20	309	310.106	44.069	148.105	100:6
Methadone	25	309	310.156	265.166	105.027	100:34
Norclozapine	50	312	313.096	192.062	270.081	100:70
Olanzapine	50	312	313.124	256.070	198.025	100:49
Clomipramine	20	314	315.120	86.087	58.072	100:37
Ranitidine	500	314	315.124	176.061	102.026	100:53
delta9-THC	10	314	315.167	193.130	123.032	100:65
Clonazepam	10	315	316.000	270.156	214.002	100:36
Oxycodone	20	315	316.115	241.094	256.128	100:82
Brompheniramine	25	318	319.060	274.026	167.065	100:52
Chlorpromazine	50	318	319.084	86.090	58.075	100:46
Fluvoxamine	20	318	319.126	71.047	200.007	100:24

Table 23: *Using an ESI spray source, targets were analyzed at 1000 ng/mL in solution for the transitions used in the analysis of other targets which share the same parent ion. S:B less than 3:1 indicated that the target would not interfere with the channels scanned for the other target, while a greater signal routed targets through additional interference testing. The following 25 targets were found to have S:B greater than 3:1 for the transitions recorded below*

Target Present in Neat Solution	Target whose fragments were scanned for	SRM		AUC	S:B
Methylphenidate	Normeperdine	324 -> 56	Blank	5.4E+03	8586
			Interference	4.6E+07	
Lamotrigine	Diphenhydramine	256 -> 165	Blank	8.0E+03	1201
			Interference	9.6E+06	
Ranitidine	Clomipramine	315 -> 86	Blank	6.2E+04	4
			Interference	2.5E+05	
		315 -> 58	Blank	2.6E+04	90
			Interference	2.3E+06	
Methadone	Fluoxetine	310 -> 44	Blank	6.0E+01	64
			Interference	3.8E+03	
		310 ->148	Blank	3.0E+01	6
			Interference	1.9E+02	
Normeperidine	Methylphenidate	234 -> 84	Blank	5.6E+03	5
			Interference	2.5E+04	
		234 ->56	Blank	3.4E+03	7478
			Interference	2.5E+07	
Pentazocine	7-Aminoclonazepam	286 -> 94	Blank	1.8E+03	63
			Interference	1.1E+05	
		286 -> 121	Blank	4.2E+03	497
			Interference	2.1E+06	
	Morphine	286 -> 152	Blank	1.0E+03	3059
			Interference	3.2E+06	
		286 -> 165	Blank	1.7E+03	77
			Interference	1.3E+05	
Normeperidine	Methylphenidate	234 -> 84	Blank	5.4E+03	5
			Interference	2.5E+04	
		234 -> 56	Blank	3.4E+03	7478
			Interference	2.5E+07	

Table 23 Continued

Target Present in Neat Solution	Target whose fragments were scanned for	SRM		AUC	S:B
Benzotropine	Zolpidem	308 -> 92	Blank	1.8E+03	54
			Interference	9.6E+04	
		308 -> 44	Blank	1.5E+03	38
			Interference	5.7E+04	
Morphine	7-Aminoclonazepam	286 -> 94	Blank	9.0E+02	140
			Interference	1.3E+05	
		286 -> 121	Blank	2.0E+03	327
			Interference	6.7E+05	
Oxycodone	Clonazepam	316 -> 270	Blank	1.4E+04	74
			Interference	1.0E+06	
		316 -> 214	Blank	2.7E+03	123
			Interference	3.3E+05	
Norpropoxyphene	Benzotropine	308 -> 165	Blank	6.6E+05	5
			Interference	3.1E+06	
Bromopheniramine	Fluvoxamine	319 ->71	Blank	2.8E+05	3
			Interference	9.9E+05	
		319 -> 200	Blank	6.2E+04	4
			Interference	2.2E+05	
Chlorpromazine	Brompheniramine	319 -> 274	Blank	4.1E+05	52
			Interference	2.1E+07	
		319 -> 167	Blank	2.2E+05	23
			Interference	5.1E+06	
	Fluvoxamine	319 -> 71	Blank	2.7E+05	7
			Interference	1.9E+06	
		319 -> 200	Blank	6.0E+04	5
			Interference	3.0E+05	
Norclozapine	Olanzapine	313 -> 256	Blank	2.8E+02	3978
			Interference	1.1E+06	
		313 -> 198	Blank	7.7E+01	229
			Interference	1.8E+04	
7-Aminoclonazepam	Morphine	286 -> 152	Blank	3.2E+04	52
			Interference	1.7E+06	
		286 -> 165	Blank	2.5E+04	6
			Interference	1.4E+05	

Table 23 Continued

Target Present in Neat Solution	Target whose fragments were scanned for	SRM		AUC	S:B
Chlordiazepoxide	Metoclopramide	300 -> 227	Blank	5.7E+05	300
			Interference	1.7E+08	
Clonazepam	Oxycodone	316 -> 241	Blank	1.1E+03	941
			Interference	1.1E+06	
		316 -> 212	Blank	1.1E+03	13
			Interference	1.4E+04	
Desalkylflurazepam	Bupivicaine	289 -> 140	Blank	1.5E+03	11121
			Interference	1.7E+07	
		289 -> 98	Blank	1.4E+03	3
			Interference	4.7E+03	
Cyclobenzapirine	MDPV	276 -> 175	Blank	3.1E+03	11
			Interference	3.4E+04	
		276 -> 135	Blank	3.5E+03	3
			Interference	1.1E+04	
Cabamazepine, 10-,11-epoxide	Phenytoin	253 -> 182	Blank	3.5E+02	25237
			Interference	8.9E+06	
		253 -> 104	Blank	3.8E+02	119
			Interference	4.5E+04	
Clomipramine	Ranitidine	315 ->176	Blank	3.6E+03	8
			Interference	2.9E+04	
		315 -> 102	Blank	7.0E+02	36
			Interference	2.5E+04	
Fluvoxamine	Brompheniramine	319 -> 274	Blank	2.5E+03	21
			Interference	5.2E+04	
		319 -> 167	Blank	5.2E+03	7
			Interference	3.5E+04	
	Chlorpromazine	319 -> 58	Blank	7.3E+03	5
			Interference	3.8E+04	
Mirtazapine	Nordoxepin	266 -> 107	Blank	1.7E+03	1196
			Interference	2.0E+06	
		266 ->235	Blank	3.0E+02	47460
			Interference	1.4E+07	

Table 23 Continued

Target Present in Neat Solution	Target whose fragments were scanned for	SRM		AUC	S:B
Nordoxepin	Mirtazapine	266 -> 195	Blank	1.3E+06	12
			Interference	1.5E+07	
		266 -> 194	Blank	7.0E+05	14
			Interference	9.8E+06	

In the next round of testing, the 25 targets identified using the ESI spray source were tested using paper spray. Targets sharing the same parent ion were combined at 100ppb in the spray solvent (95:5:0.01 methanol:water:acetic acid) and sprayed over blank paper. All of the fragment ions were scanned and targets ratios were monitored to see if the presence of the interfering compound caused the target's ratio to vary more than the permitted ±30%. The results of these experiments, found in Table 24, indicate that out of the 20 target pairs/groups, only 9 targets may produce false negatives in the presence of another target that shares a parent ion with it. These targets are: phenytoin, norsertraline, desalkylflurazepam, chlordizepoxide, metoclopramide, zaleplon, benztropine, fluoxetine, and olanzapine. The transitions belonging to methadone were unintentionally left out of this series of tests, and no conclusion is made about the affect of the presence of fluoxetine on methadone at this time.

Table 24 *Targets with the same parent ion were each added at 100 ng/mL to the spray solvent and sprayed over blank paper. The fragmentation ion for each target was monitored and those that differed from their established ratio by greater than the permissible deviation of ±30% were flagged, as shown in red. The remaining targets were concluded to not interfere with one another*

Name	Parent Ion	Product Ion 1	Peak Area	Product Ion 2	Peak Area	Ratio	Ratio Deviation (%)
Primidone	219	162	2.77E+06	91	1.99E+06	72	2.7
Meprobamate	219	158	1.21E+06	97	1.24E+06	102	1.2
Amobarbital	225	182	1.27E+08	42	6.12E+07	48	0.0
Pentobarbital	225	182	1.27E+08	42	6.12E+07	48	0.1
Normeperidine	234	160	4.80E+07	56	1.22E+07	25	15.3
Methylphenidate	234	84	8.50E+07	56	1.22E+07	14	19.3
Phenytoin	253	182	2.75E+06	104	5.44E+05	20	**-69.6**
Carbamazepine-10,11-epoxide	253	180	1.50E+07	210	5.69E+06	38	5.6
Lamotrigine	256	211	1.48E+07	145	9.20E+06	62	-4.2
Diphenhydramine	256	167	8.80E+07	165	3.98E+07	45	-5.8
Carisoprodol	261	97	1.56E+06	55	1.31E+06	84	3.7
Ropinirole	261	114	1.31E+08	86	3.03E+07	23	-7.4
Nordoxepin	266	107	6.79E+07	235	4.25E+07	63	9.8
Mirtazapine	266	195	1.85E+08	194	7.06E+07	38	-6.8
Norsertraline	275	159	1.99E+07	124	1.41E+06	7	**-72.7**
Chlorpheniramine	275	230	1.40E+08	167	7.26E+07	52	-4.3
MDPV	276	175	7.22E+07	135	6.56E+07	91	-0.2
Cyclobenzaprine	276	215	1.83E+08	216	1.01E+08	55	-1.6
EDDP	278	234	2.90E+08	249	1.16E+08	40	-21.7
Venlafaxine	278	58	6.41E+07	121	1.65E+07	26	-1.3
7-aminoclonazepam	286	222	1.13E+07	121	9.02E+06	80	-5.8
Morphine	286	152	6.45E+06	165	3.92E+06	61	-13.1
Pentazocine	286	218	1.08E+10	69	2.46E+09	23	-21.7
Desalkylflurazepam	289	140	2.02E+08	226	4.84E+06	2	**-96.4**
Bupivacaine	289	140	2.02E+08	98	4.34E+07	21	12.8
Chlordiazepoxide	300	227	2.88E+08	241	3.14E+07	11	**-59.7**
Metoclopramide	300	227	6.70E+10	184	8.86E+07	0	**-99.7**
Sertraline	306	159	5.43E+07	275	4.55E+07	84	-0.2
Zaleplon	306	236	9.78E+06	264	5.16E+06	53	**-37.9**

Table 24 continued

Name	Parent Ion	Product Ion 1	Peak Area	Product Ion 2	Peak Area	Ratio	Ratio Deviation (%)
Zolpidem	308	235	3.17E+08	92	5.71E+07	18	-5.4
Benztropine	308	167	3.92E+08	165	1.81E+08	46	**-32.0**
Norpropoxyphene	308	100	2.40E+07	44	1.61E+07	67	-5.1
Fluoxetine	310	44	1.83E+07	148	2.32E+06	13	**111.8**
Methadone	310	265	-	105	7.04E+07	-	-
Norclozapine	313	192	1.24E+08	270	8.86E+07	72	2.4
Olanzapine	313	256	1.99E+07	198	5.39E+06	27	**-44.7**
Clomipramine	315	86	1.06E+08	58	3.78E+07	36	-3.3
Ranitidine	315	176	4.24E+07	102	2.22E+07	52	-1.3
Clonazepam	316	270	1.19E+07	214	3.83E+06	32	-10.8
Oxycodone	316	241	1.53E+07	256	1.15E+07	75	-8.3
Brompheniramine	319	274	1.29E+08	167	6.25E+07	48	-7.1
Chlorpromazine	319	86	1.20E+08	58	5.35E+07	45	-2.9
Fluvoxamine	319	71	2.57E+07	200	5.51E+06	21	-10.6

6.3 Conclusions and Future Work

Using database searches, 540 compounds were found who could potentially interfere with the target analytes of this study. They would only be true interferences though, if they exist at detectable concentrations, ionize via protonation and produce a daughter fragment with the same mass to charge ratio as one that is monitored for one of the analytical targets.

Nine targets were identified who could interfere with other targets and provide false negatives by skewing the fragmentation ratios outside of the permitted ±30% deviation. Carbamazepine-10,11-epoxide was identified as a potential interference for phenytoin, and chlorphenirameine was identified as a potential interferences for norsertraline. Bupivacaine was identified as a potential interference to desalkylflurazepam, which is to be assumed to be a true interference since both analytes monitor the fragment with m/z 140. Likewise, chlordiazepoxide and metoclopramide interfere with one another because they both share the fragment with m/z 227. Sertraline was identified as a potential

interference for zaleplon, and methadone was identified as a potential interference to fluoxetine. Finally, norclozapine was identified as a potential interference to the detection of olanzapine.

A final set of experiments needs to be run to confirm the effect of intra-target interferences. The nine analytes that were identified as potential interferences in neat solution need to be analyzed in blood along with the targets that seem to cause their fragmentation ratios to skew. The extraction efficiencies and the ionization suppression will likely be different for each target, so the targets should be run together with the interfering compound at a very high concentration to test for a "worst case scenario," and they should also be run at the cutoff levels for a more realistic assessment of the extent to which they may interfere.

7 CONCLUSIONS

Drug screening is a necessary tool in postmortem investigations that focuses analytical efforts, time, and resources. Currently, drug screening itself can consume huge amounts of time and money in sample clean-up and analysis, and adding newly emerging compounds can be cumbersome. Paper Spray Mass Spectrometry provides an alternative method for drug screening that drastically simplifies the screening process, allowing for rapid detection of a wide variety of drugs. While paper spray analysis has historically been applied to a small focused panel of targets, by adapting existing techniques, it has been found to be suitable to use in large-panel screening as well.

7.1 Summary of Conclusions

Two methods were developed during this project: the first was a manually run method that used hand-cut paper. By detecting two daughter fragments and by monitoring the ratio between their relative abundancies, this method was able to detect 114 drugs and metabolites at toxicologically relevant concentrations in blood. Using this method, screening the entire panel of drugs can be accomplished in three sixty second runs, requiring only 10.5 µL of blood. Screening these 114 targets, this "manual" method would require approximately 9 minutes total analysis time. In order to really make paper spray a viable alternative, a second, more automated and commercially available method was developed as well.

The second method used commercially available disposable cartridges on an automated ionization source. Under the same detection criteria used in the manually run experiments, this method was able to successfully detect a panel of 100 drugs and metabolites at toxicologically relevant concentrations in blood. This method would also require 3 replicate dried blood spot samples due to the time needed for the mass spectrometer to scan through all of the SRM channels. Because of the larger paper size, this method uses almost four times as much blood as the manual method, but the total volume of blood required is still less than 50 µL. The automated method has the same analytical run time

as the manual method, however, there is very little down time between samples since the cartridges do not have to be cleaned, so the entire screening process can be accomplished in about 4 minutes.

In addition to the successively developed methods, a list of potentially interfering compounds was compiled that can inform future data interpretation. Within the analytes on the panel tested, 9 were identified that could potentially interfere with one another and lead to false negatives if both a present in a sample.

7.2 Discussion

The target levels of detection used in developing these methods were based on the lower limit of quantitation for methods used at a local toxicology laboratory. While the level of detection for paper spray was found to be higher than the LLOQ presented by the toxicology laboratory in some instances, other times targets were run at lower concentrations than the reported LLOQ's. In the instances where PS-MS was not sufficiently sensitive, it will be important to determine if the LOD is at a toxicologically relevant concentration; in the cases where it is more sensitive, paper spray could help properly route samples into confirmatory testing which could potentially be false negatives when analyzed using other methods.

The concentrations, whether run at proposed cutoff levels or experimental LOD's, used during analysis were established in method development using the manual method. The higher number of target analytes that were ultimately able to be detected in the manual method vs the automated method using the laser-cut paper suggest room for further method optimization and development for the automated method. Although only about half as many targets were analyzed on die-cut paper as laser-cut paper, the percent of targets detected on the die-cut paper (87%) is much more congruent with the manual method (86%) than the laser-cut paper is (76%). It is possible that the laser cutting approach used during manufacturing requires some refinement. If this is the case, a new LOD may need to be established using the laser-cut paper, especially in the 11 cases where a target was detected using the manual method, but not using the automated

method with the laser-cut paper. Revisiting blood spot placement and dilution could also prove advantageous for future experiments run on the laser-cut paper.

In this project, 14 of the originally proposed analytical targets were not included because preliminary tests showed that they only ionized well in negative mode and would require further optimization in order to reach acceptable limits of detection. This work was completed in a separate project, by McKenna et. al. where an optimized solvent of 90:10:0.01 MeOH:CCl_4:NH_4OH was used to successfully detect all 14 targets, 4 of which were determined to have LOD's slightly higher than the purposed screening cutoff concentrations.[67] This work was done on an exact mass instrument, but would be translated to a triple quadrupole instrument.

7.3 Future work

The next set of experiments that need to be run are establishing calibration curves for each of the analytes. This will establish an experimental LOD and can help inform acceptance criteria. One of the largest hurdles faced in this project was establishing a permissible ratio tolerance. Ratio tolerances are always discussed in the context of chromatography, and usually at concentrations well above the LOD. Since no intentional chromatography is performed during PS and this project deals with some analytes near or at their LOD, running calibration curves will help establish the robustness of fragment ion ratios in a context that has yet to be established. This could lead to justifying either broadening or constricting the current ±30% permissible deviation, or even a ratio tolerance that is tied to signal to blank ratio. To run a set of calibration curves, internal standards (IS) will need to be chosen. Since it is impractical to use a stable-labeled internal standard for every target, work will need to be done to find a reasonable number of analogue standards that can act as internal standards for the entire panel of targets. Ideally, these IS's could be cocktailed together and spiked directly into a sample.

In addition to running calibration curves, a final blinded study of postmortem samples should be run to determine the effectiveness of the assay on true postmortem samples. These will included blood in various degrees of decomposition and can be used to determine the false positive and false negative rate of the assay.

BIBLIOGRAPHY

1. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. European Commission; 2002.
2. Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. *Critical Care.* 2012;16(4):R136-R136.
3. Directorate ECHaCP. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. . 2013.
4. Borzelleca JF. Paracelsus: Herald of Modern Toxicology. *Toxicol Sci.* 2000;53(1):2-4.
5. Committee WL. *WADA Technical Document – TD2010IDCR.* World Antidoping Agency;2010.
6. European Laboratory Guidelines for Legally Defensible Workplace Drug Testing Version 1.0. 2002; 39.
7. Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens. 2009.
8. SWGTOX. Scientific Working Group for Forensic Toxicology (SWGTOX) Standard for Mass Spectral Data Acceptance for Definitive Identification. swgtox.org. http://www.swgtox.org/documents/PublicComment20140715.pdf. Published July 15, 2015. Accessed August 8, 2016.
9. Logan BK, Goldberger BA, Caplan YH, et al. *What is Forensic Toxicology?* July 2010.
10. Ojanperä I, Kolmonen M, Pelander A. Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control. *Analytical and Bioanalytical Chemistry.* 2012;403(5):1203-1220.
11. Bogusz MJ. *Forensic Science.* Elsevier Science; 2000.

12. Stimpfl T, Muller K, Gergov M, et al. *Recommendations on Sample Preparation of Biological Specimens for Systematic Toxicological Analysis.* The International Association of Forensic Toxicologists.
13. Lappas NT, Lappas CM. *Forensic Toxicology: Principles and Concepts.* Elsevier Science; 2015.
14. Jarvis M. Simultaneous Targeted and Unknown Screening using the 3200 QTRAP LC/MS/MS System and Cliquid Software. In: AB SCIEX C, ed: AB SCIEX; 2011.
15. Hearn WL, Jones GR, McCutcheon JR, Logan BK, Middleberg RA. *Forensic Toxicology Laboratory Guidelines.* Society of Forensic Toxicologists/American Academy of Forensic Sciences; February 23, 2006.
16. Manicke NE. NIJ 2014 Program Narrative. Indiana University-Purdue University Indianapolis; 2014:45.
17. Liu J, Wang H, Manicke NE, Lin J-M, Cooks RG, Ouyang Z. Development, Characterization, and Application of Paper Spray Ionization. *Anal Chem.* 2010;82(6):2463-2471.
18. Manicke NE, Bills BJ, Zhang C. Analysis of biofluids by paper spray MS: advances and challenges. *Bioanalysis.* 2016;8(6):589-606.
19. Takyi-Williams J, Dong X, Gong H, et al. Application of paper spray-MS in PK studies using sunitinib and benzethonium as model compounds. *Bioanalysis.* 2015;7(4):413-423.
20. Evard H, Kruve A, Lõhmus R, Leito I. Paper spray ionization mass spectrometry: Study of a method for fast-screening analysis of pesticides in fruits and vegetables. *Journal of Food Composition and Analysis.* 2015;41:221-225.
21. Li A, Wei P, Hsu HC, Cooks RG. Direct analysis of 4-methylimidazole in foods using paper spray mass spectrometry. *Analyst.* 2013;138(16):4624-4630.
22. Taverna D, Di Donna L, Mazzotti F, Policicchio B, Sindona G. High-throughput determination of Sudan Azo-dyes within powdered chili pepper by paper spray mass spectrometry. *J Mass Spectrom.* 2013;48(5):544-547.

23. Soparawalla S, Tadjimukhamedov FK, Wiley JS, Ouyang Z, Cooks RG. In situ analysis of agrochemical residues on fruit using ambient ionization on a handheld mass spectrometer. *Analyst.* 2011;136(21):4392-4396.
24. Wang H, Manicke NE, Yang Q, et al. Direct analysis of biological tissue by paper spray mass spectrometry. *Anal Chem.* 2011;83(4):1197-1201.
25. Oradu SA, Cooks RG. Multistep mass spectrometry methodology for direct characterization of polar lipids in green microalgae using paper spray ionization. *Anal Chem.* 2012;84(24):10576-10585.
26. Hamid AM, Jarmusch AK, Pirro V, et al. Rapid discrimination of bacteria by paper spray mass spectrometry. *Anal Chem.* 2014;86(15):7500-7507.
27. Jain S, Heiser A, Venter AR. Spray desorption collection: an alternative to swabbing for pharmaceutical cleaning validation. *Analyst.* 2011;136(7):1298-1301.
28. Berm EJ, Paardekooper J, Brummel-Mulder E, Hak E, Wilffert B, Maring JG. A simple dried blood spot method for therapeutic drug monitoring of the tricyclic antidepressants amitriptyline, nortriptyline, imipramine, clomipramine, and their active metabolites using LC-MS/MS. *Talanta.* 2015;134:165-172.
29. Lee H, Jhang CS, Liu JT, Lin CH. Rapid screening and determination of designer drugs in saliva by a nib-assisted paper spray-mass spectrometry and separation technique. *J Sep Sci.* 2012;35(20):2822-2825.
30. Su Y, Wang H, Liu J, Wei P, Cooks RG, Ouyang Z. Quantitative paper spray mass spectrometry analysis of drugs of abuse. *Analyst.* 2013;138(16):4443-4447.
31. Zhang Z, Xu W, Manicke NE, Cooks RG, Ouyang Z. Silica coated paper substrate for paper-spray analysis of therapeutic drugs in dried blood spots. *Anal Chem.* 2012;84(2):931-938.
32. Manicke NE, Yang Q, Wang H, Oradu S, Ouyang Z, Cooks RG. Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots. *Int J Mass spectrom.* 2011;300(2-3):123-129.
33. Wang H, Ren Y, McLuckey MN, et al. Direct quantitative analysis of nicotine alkaloids from biofluid samples using paper spray mass spectrometry. *Anal Chem.* 2013;85(23):11540-11544.

34. Espy RD, Teunissen SF, Manicke NE, et al. Paper spray and extraction spray mass spectrometry for the direct and simultaneous quantification of eight drugs of abuse in whole blood. *Anal Chem.* 2014;86(15):7712-7718.
35. Manicke NE, Abu-Rabie P, Spooner N, Ouyang Z, Cooks RG. Quantitative Analysis of Therapeutic Drugs in Dried Blood Spot Samples by Paper Spray Mass Spectrometry: An Avenue to Therapeutic Drug Monitoring. *J Am Soc Mass Spectrom.* 2011;22(9):1501-1507.
36. Yang Q, Wang H, Maas JD, et al. Paper spray ionization devices for direct, biomedical analysis using mass spectrometry. *Int J Mass spectrom.* 2012;312:201-207.
37. Shen L, Zhang J, Yang Q, Manicke NE, Ouyang Z. High throughput paper spray mass spectrometry analysis. *Clin Chim Acta.* 2013;420:28-33.
38. Espy RD, Manicke NE, Ouyang Z, Cooks RG. Rapid analysis of whole blood by paper spray mass spectrometry for point-of-care therapeutic drug monitoring. *Analyst.* 2012;137(10):2344-2349.
39. Potter R, Manicke N, Shanks K. NIJ Project Consultation. August 2016.
40. Quimby B. Improved Forensic Toxicology Screening Using A GC/MS/NPD System with a 725-Compound DRS Database. *Agilent Technologies: Application.* 2008:28.
41. Chen D, Yu J, Tao Y, et al. Qualitative screening of veterinary anti-microbial agents in tissues, milk, and eggs of food-producing animals using liquid chromatography coupled with tandem mass spectrometry. *J Chromatogr B.* 2016;1017–1018:82-88.
42. Han S, Zhou T, Yin B, He P. A sensitive and semi-quantitative method for determination of multi-drug residues in animal body fluids using multiplex dipstick immunoassay. *Anal Chim Acta.* 2016;927:64-71.
43. Alfazil AA, Anderson RA. Stability of benzodiazepines and cocaine in blood spots stored on filter paper. *J Anal Toxicol.* 2008;32(7):511-515.
44. Wagner M, Tonoli D, Varesio E, Hopfgartner G. The use of mass spectrometry to analyze dried blood spots. *Mass Spectrom Rev.* 2016;35(3):361-438.

45. Kushnir MM, Rockwood AL, Nelson GJ, Yue B, Urry FM. Assessing analytical specificity in quantitative analysis using tandem mass spectrometry. *Clin Biochem.* 2005;38(4):319-327.
46. Kaufmann A, Widmer M, Maden K. Signal suppression can bias selected reaction monitoring ratios. Implications for the confirmation of positive findings in residue testing. *Rapid Commun Mass Spectrom.* 2014;28(8):899-907.
47. Sauvage FL, Gaulier JM, Lachatre G, Marquet P. Pitfalls and prevention strategies for liquid chromatography-tandem mass spectrometry in the selected reaction-monitoring mode for drug analysis. *Clin Chem.* 2008;54(9):1519-1527.
48. Niessen WMA. Fragmentation of toxicologically relevant drugs in positive-ion liquid chromatography–tandem mass spectrometry. *Mass Spectrom Rev.* 2011;30(4):626-663.
49. Mol HG, Zomer P, Garcia Lopez M, et al. Identification in residue analysis based on liquid chromatography with tandem mass spectrometry: Experimental evidence to update performance criteria. *Anal Chim Acta.* 2015;873:1-13.
50. Kaufmann A, Butcher P, Maden K, Widmer M, Giles K, Uria D. Are liquid chromatography/electrospray tandem quadrupole fragmentation ratios unequivocal confirmation criteria? *Rapid Commun Mass Spectrom.* 2009;23(7):985-998.
51. Vogeser M, Seger C. Pitfalls Associated with the Use of Liquid Chromatography–Tandem Mass Spectrometry in the Clinical Laboratory. *Clin Chem.* 2010;56(8):1234-1244.
52. Lynch KL, Breaud AR, Vandenberghe H, Wu AH, Clarke W. Performance evaluation of three liquid chromatography mass spectrometry methods for broad spectrum drug screening. *Clin Chim Acta.* 2010;411(19-20):1474-1481.
53. Saar E, Beyer J, Gerostamoulos D, Drummer OH. The time-dependant post-mortem redistribution of antipsychotic drugs (vol 222, pg 223, 2012). *Forensic Science International.* 2013;228(1-3):94-94.

54. Saar E, Gerostamoulos D, Drummer OH, Beyer J. Comparison of extraction efficiencies and LC–MS–MS matrix effects using LLE and SPE methods for 19 antipsychotics in human blood. *Analytical & Bioanalytical Chemistry.* 2009;393(2):727-734.

55. Rosano TG, Wood M, Swift TA. Postmortem Drug Screening by Non-Targeted and Targeted Ultra-Performance Liquid Chromatography-Mass Spectrometry Technology. *J Anal Toxicol.* 2011;35(7):411-423.

56. Peters F, Remane D. Aspects of matrix effects in applications of liquid chromatography-mass spectrometry to forensic and clinical toxicology-a review. *Analytical & Bioanalytical Chemistry.* 2012;403(8):2155-2172.

57. Zhang C, Manicke NE. Development of a Paper Spray Mass Spectrometry Cartridge with Integrated Solid Phase Extraction for Bioanalysis. *Anal Chem.* 2015;87(12):6212-6219.

58. Cullum N, Meng C-K, Zavitsanos P. Effect of Sample Matrix on Suppression of Ionization in Water Samples Using LC-ESI-MS. *Agilent Technologies: Application Note.* 2004:10. Accessed June 27, 2016.

59. Vega C, Spence C, Zhang C, Bills BJ, Manicke NE. Ionization Suppression and Recovery in Direct Biofluid Analysis Using Paper Spray Mass Spectrometry. *J Am Soc Mass Spectrom.* 2016;27(4):726-734.

60. Shi RZ, El Gierari el TM, Manicke NE, Faix JD. Rapid measurement of tacrolimus in whole blood by paper spray-tandem mass spectrometry (PS-MS/MS). *Clin Chim Acta.* 2015;441:99-104.

61. Warnke S, Seo J, Boschmans J, et al. Protomers of Benzocaine: Solvent and Permittivity Dependence. *J Am Chem Soc.* 2015;137(12):4236-4242.

62. Wang J, Aubry A, Bolgar MS, et al. Effect of mobile phase pH, aqueous-organic ratio, and buffer concentration on electrospray ionization tandem mass spectrometric fragmentation patterns: implications in liquid chromatography/tandem mass spectrometric bioanalysis. *Rapid Commun Mass Spectrom.* 2010;24(22):3221-3229.

63. Wishart DS, Jewison T, Guo AC, et al. HMDB 3.0--The Human Metabolome Database in 2013. *Nucleic Acids Res.* 2013;41(Database issue):D801-807.

64. Wishart DS, Knox C, Guo AC, et al. HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res.* 2009;37(Database issue):D603-610.

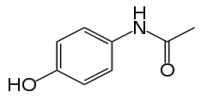
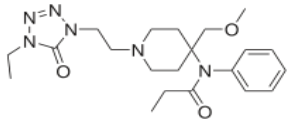
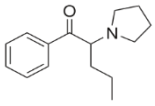
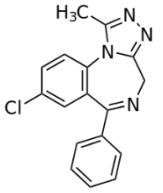
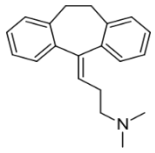
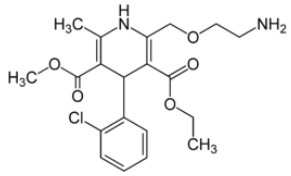
65. Wishart DS, Tzur D, Knox C, et al. HMDB: the Human Metabolome Database. *Nucleic Acids Res.* 2007;35(Database issue):D521-526.

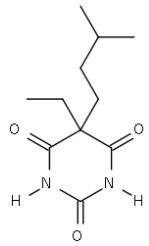
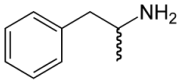
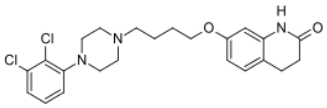
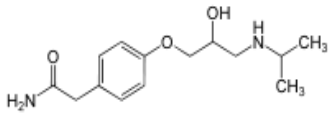
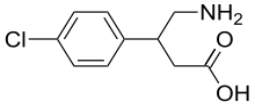
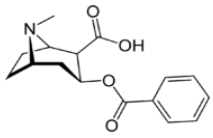
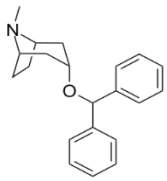
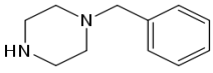
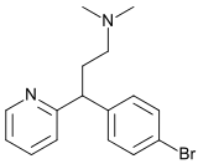
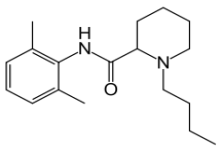
66. Wishart DS, Knox C, Guo AC, et al. DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res.* 2006;34(Database issue):D668-672.

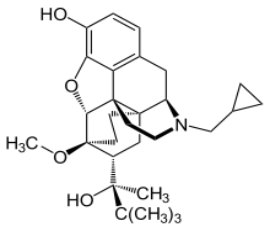
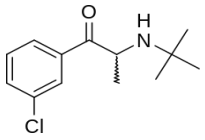
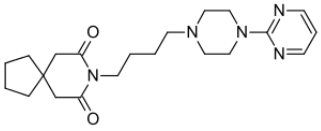
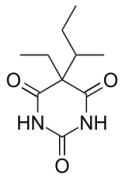
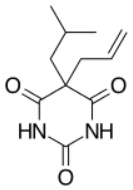
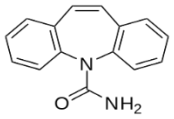
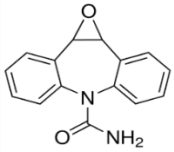
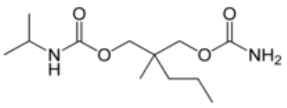
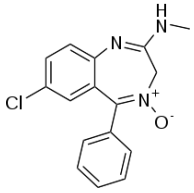
67. McKenna J, Dhummakupt ES, Connell T, Demond PS, Miller DB, Nilles JM, Manicke NE and Glaros T: Detection of Chemical Warfare Agent Simulants and Hydrolysis Products in Biological Samples by Paper Spray Mass Spectrometry. Analyst. In Press

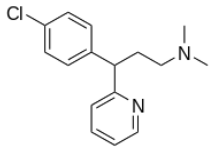
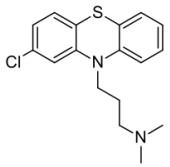
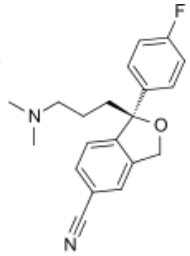
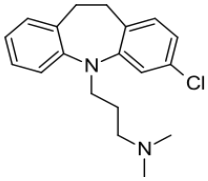
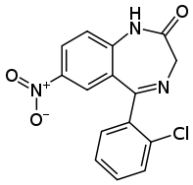
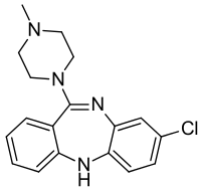
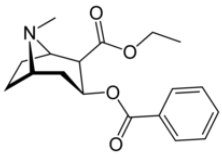
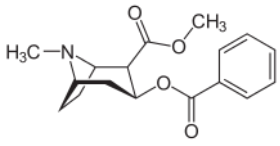
APPENDIX A

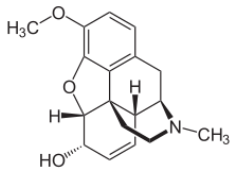
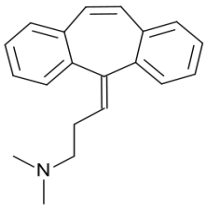
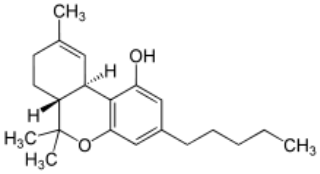
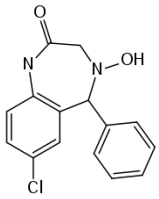
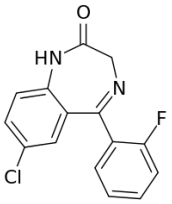
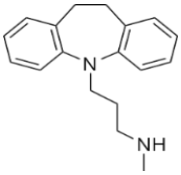
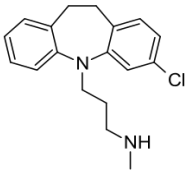
Purposed Analytical Targets with Cutoff Values from AFT

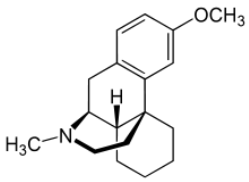
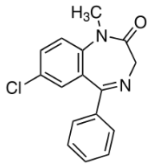
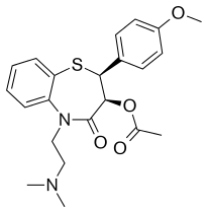
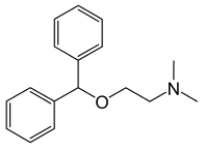
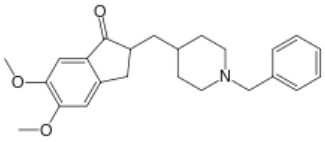
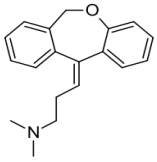
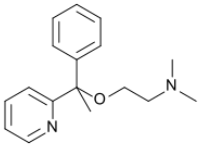
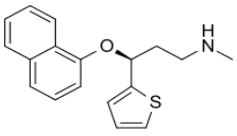
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Acetaminophen	Analgesic	20000	
Alfentanil	Narcotic	50	
Alpha-PVP	Stimulant	50	
Alprazolam	Benzodiazepine	5	
Amitriptyline	Antidepressant	20	
Amlodipine	Cardiovascular	20	

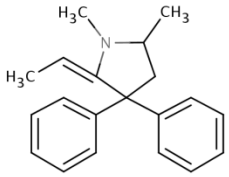
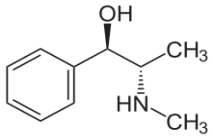
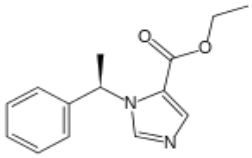
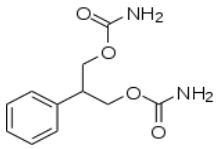
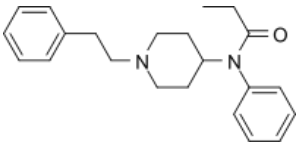
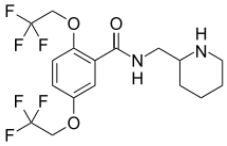
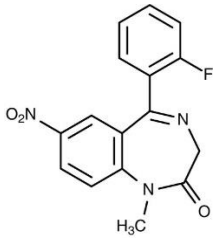
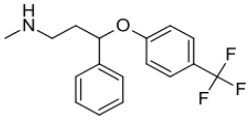
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Amobarbital	Barbiturate	500	
Amphetamine	Amphetamine	50	
Aripiprazole	Antipsychotic	50	
Atenolol	Cardiovascular	100	
Baclofen	Analgesic	1000	
Benzoylecgonine	Cocaine	50	
Benztropine	Neurological	10	
Benzylpiperazine	Miscellaneous	50	
Brompheniramine	Antihistamine	25	
Bupivacaine	Anesthetic	500	

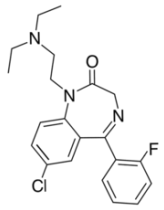
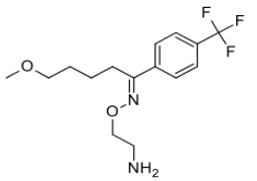
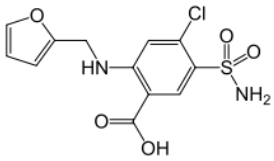
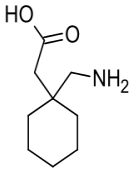
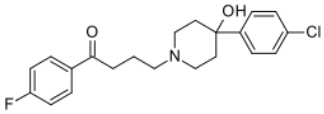
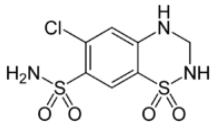
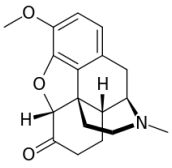
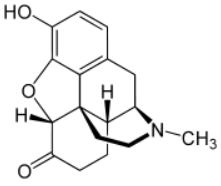
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Buprenorphine	Analgesic	1	
Bupropion	Antidepressant	50	
Buspirone	Antipsychotic	10	
Butabarbital	Barbiturate	500	
Butalbital	Barbiturate	500	
Carbamazepine	Anticonvulsant	1000	
Carbamazepine-10,11-epoxide	Anticonvulsant	1000	
Carisoprodol	Analgesic	2000	
Chlordiazepoxide	Benzodiazepine	50	

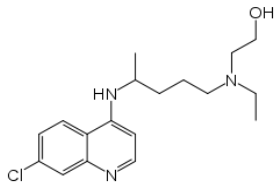
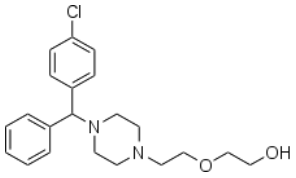
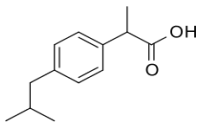
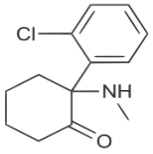
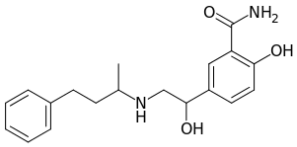
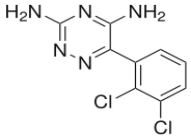
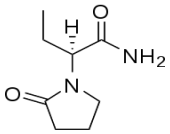
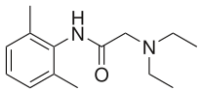
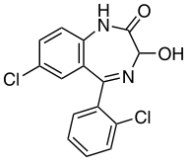
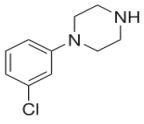
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Chlorpheniramine	Antihistamine	25	
Chlorpromazine	Antipsychotic	50	
Citalopram	Antidepressant	10	
Clomipramine	Antidepressant	20	
Clonazepam	Benzodiazepine	10	
Clozapine	Antipsychotic	50	
Cocaethylene	Cocaine	50	
Cocaine	Cocaine	50	

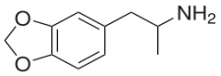
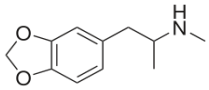
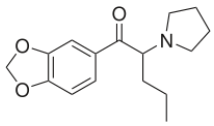
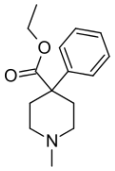
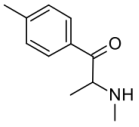
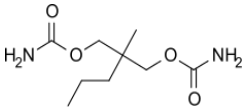
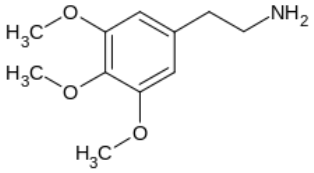
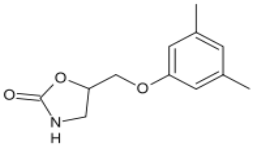
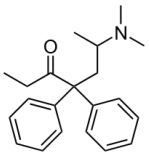
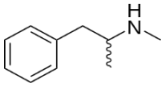
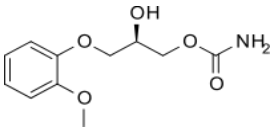
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Codeine	Opiate	20	
Cyclobenzaprine	Analgesic	10	
delta9-THC	Cannabinoid	10	
Demoxepam	Benzodiazepine	50	
Desalkylflurazepam	Benzodiazepine	50	
Desipramine	Antidepressant	20	
Desmethylclomipramine	Antidepressant	20	

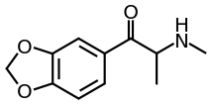
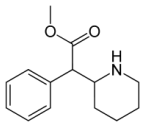
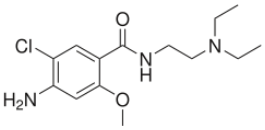
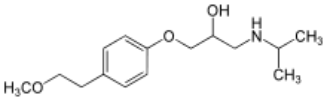
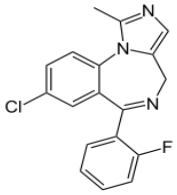
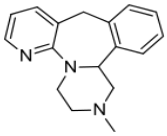
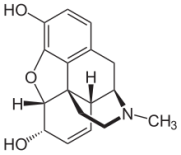
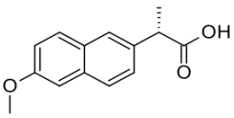
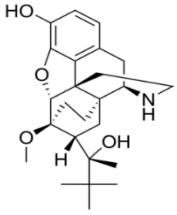
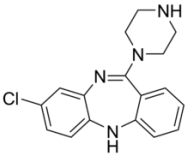
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Dextromethorphan	Narcotic	10	
Diazepam	Benzodiazepine	50	
Diltiazem	Cardiovascular	50	
Diphenhydramine	Antihistamine	25	
Donepezil	Neurological	50	
Doxepin	Antidepressant	20	
Doxylamine	Antihistamine	25	
Duloxetine	Antidepressant	20	

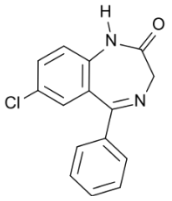
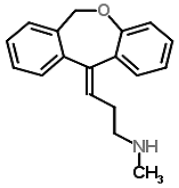
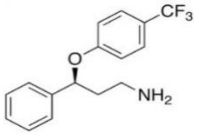
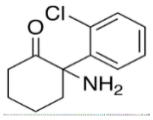
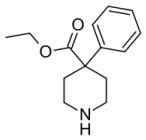
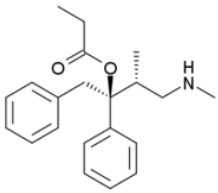
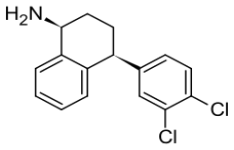
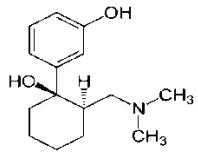
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
EDDP	Methadone	25	
Ephedrine/Pseudoephedrine	Amphetamine	50	
Etomidate	Anesthetic	100	
Felbamate	Anticonvulsant	10000	
Fentanyl	Fentanyl	1	
Flecainide	Cardiovascular	500	
Flunitrazepam	Sedative/Hypnotic	20	
Fluoxetine	Antidepressant	20	

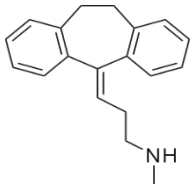
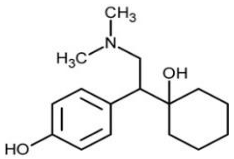
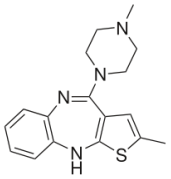
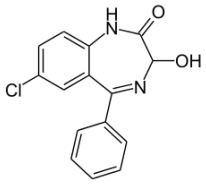
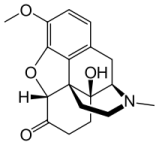
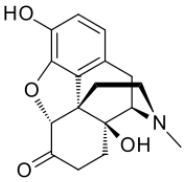
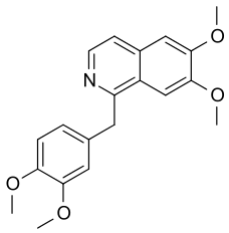
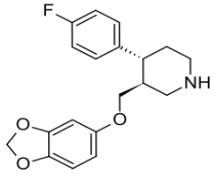
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Flurazepam	Benzodiazepine	25	
Fluvoxamine	Antidepressant	20	
Furosemide	Cardiovascular	1000	
Gabapentin	Anticonvulsant	500	
Haloperidol	Antipsychotic	10	
Hydrochlorothiazide	Cardiovascular	100	
hydrocodone	Opiate	20	
hydromorphone	Opiate	20	

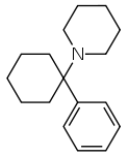
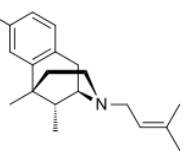
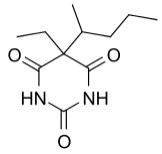
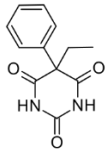
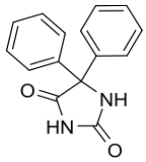
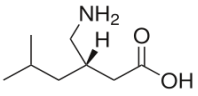
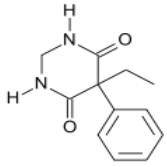
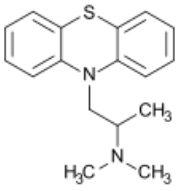
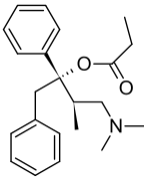
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Hydroxychloroquine	Analgesic	2000	
Hydroxyzine	Antihistamine	10	
Ibuprofen	Analgesic	10000	
Ketamine	Anesthetic	100	
Labetalol	Cardiovascular	50	
Lamotrigine	Anticonvulsant	1000	
Levetiracetam	Anticonvulsant	2000	
Lidocaine	Anesthetic	500	
Lorazepam	Benzodiazepine	25	
mCPP	Antidepressant	20	

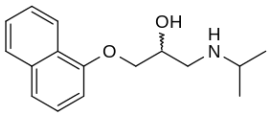
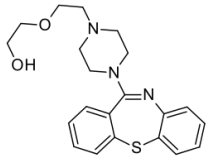
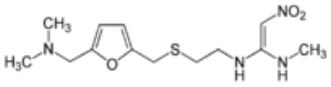
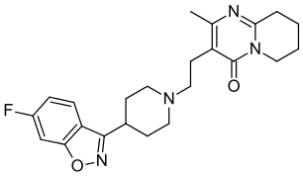
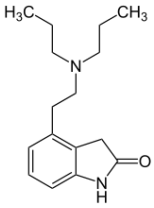
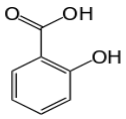
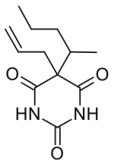
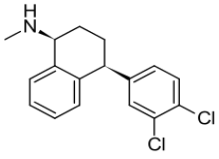
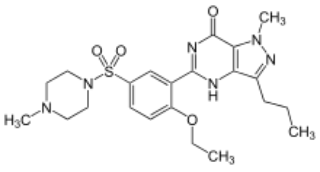
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
MDA	Amphetamine	50	
MDMA	Amphetamine	50	
MDPV	Stimulant	50	
Meperidine	Narcotic	25	
Mephedrone	Stimulant	50	
Meprobamate	Analgesic	2000	
Mescaline	Miscellaneous	50	
Metaxalone	Analgesic	1000	
Methadone	Methadone	25	
Methamphetamine	Amphetamine	50	
Methocarbamol	Analgesic	1000	

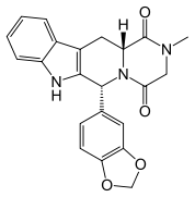
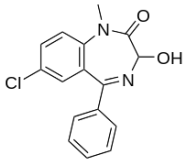
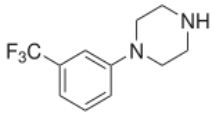
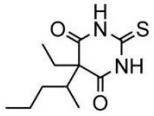
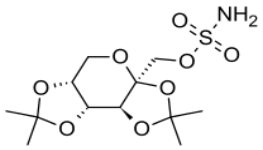
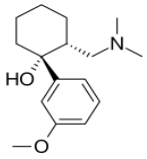
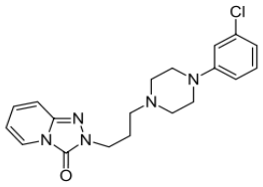
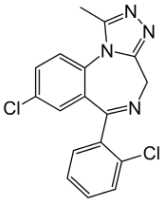
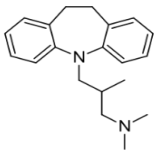
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Methylone	Stimulant	50	
Methylphenidate	Stimulant	20	
Metoclopramide	Gastrointestinal	100	
Metoprolol	Cardiovascular	50	
Midazolam	Anesthetic	50	
Mirtazapine	Antidepressant	50	
Morphine	Opiate	20	
Naproxen	Analgesic	30000	
Norbuprenorphine	Analgesic	1	
Norclozapine	Antipsychotic	50	

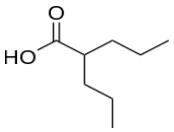
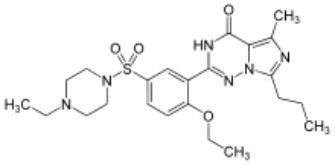
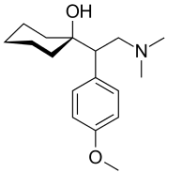
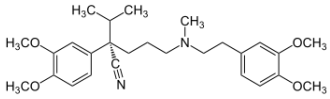
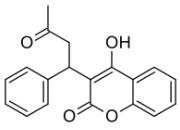
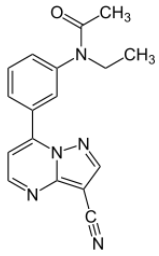
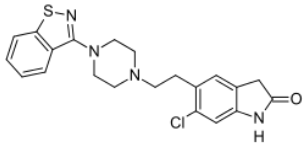
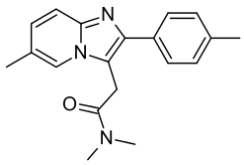
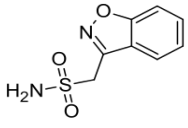
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Nordiazepam	Benzodiazepine	50	
Nordoxepin	Antidepressant	20	
Norfluoxetine	Antidepressant	20	
Norketamine	Anesthetic	100	
Normeperidine	Narcotic	25	
Norpropoxyphene	Propoxyphene	50	
Norsertraline	Antidepressant	100	
Nortramadol	Analgesic	100	

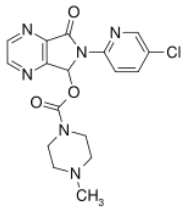
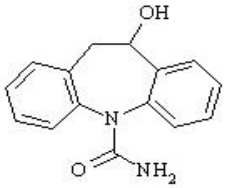
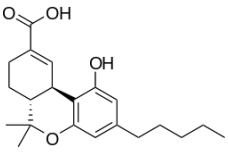
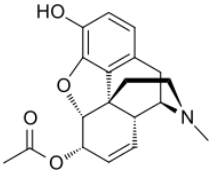
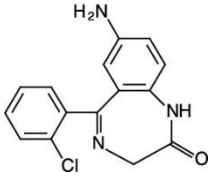
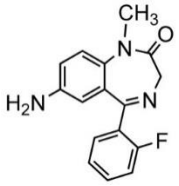
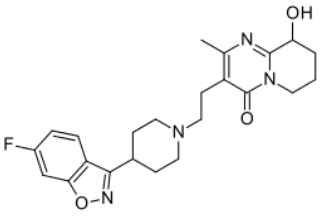
Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Nortriptyline	Antidepressant	20	
Norvenlafaxine	Antidepressant	50	
Olanzapine	Antipsychotic	50	
Oxazepam	Benzodiazepine	50	
Oxycodone	Opiate	20	
Oxymorphone	Opiate	20	
Papaverine	Cardiovascular	500	
Paroxetine	Antidepressant	20	

Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
PCP	Phencyclidine	25	
Pentazocine	Narcotics	50	
Pentobarbital	Barbiturate	500	
Phenobarbital	Barbiturate	2000	
Phenytoin	Anticonvulsant	5000	
Pregabalin	Anticonvulsant	500	
Primidone	Anticonvulsant	5000	
Promethazine	Sedative/Hypnotic	25	
Propoxyphene	Propoxyphene	50	

Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Propranolol	Cardiovascular	50	
Quetiapine	Antipsychotic	50	
Ranitidine	Gastrointestinal	500	
Risperidone	Antipsychotic	10	
Ropinirole	Neurological	10	
Salicylic Acid	Analgesic	9000	
Secobarbital	Barbiturate	500	
Sertraline	Antidepressant	100	
Sildenafil	Urological	100	

Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Tadalafil	Urological	100	
Temazepam	Benzodiazepine	50	
TFMPP	Miscellaneous	50	
Thiopental	Sedative/Hypnotic	2000	
Topiramate	Anticonvulsant	2000	
Tramadol	Analgesic	100	
Trazodone	Antidepressant	100	
Triazolam	Benzodiazepine	20	
Trimipramine	Antidepressant	20	

Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Valproic Acid	Anticonvulsant	5000	
Vardenafil	Urological	100	
Venlafaxine	Antidepressant	50	
Verapamil	Cardiovascular	50	
Warfarin	Cardiovascular	500	
Zaleplon	Sedative/Hypnotic	25	
Ziprasidone	Antipsychotic	10	
Zolpidem	Sedative/Hypnotic	10	
Zonisamide	Anticonvulsant	10000	

Target	Drug Class	Target Screening Cutoff (ng/mL)	Chemical Structure
Zopiclone	Sedative/Hypnotic	10	
10-monohydroxyoxcarbazepine	Anticonvulsant	1000	
11-nor-9-Carboxy-THC	Cannabinoid	10	
6-acetylmorphine	Opiate	20	
7-aminoclonazepam	Benzodiazepine	10	
7-aminoflunitrazepam	Sedative/Hypnotic	20	
9-hydroxyrisperidone	Antipsychotic	10	

APPENDIX B

Detailed results from loading capacity and blood dilution study for the five targets analyzed in Chapter 4.

		Morphine					
		Whole Blood	Diluted Blood	Whole Blood	Diluted Blood	Whole Blood	Diluted Blood
	Fragment	**286 -> 152**		**286 -> 165**		**286 -> 201**	
5 uL	Avg Blank	7.7E+03	5.6E+03	4.8E+03	4.7E+03	3.1E+05	3.7E+05
	Stdev	4.3E+03	1.0E+03	2.5E+03	5.3E+02	7.2E+04	4.4E+04
	Avg Drug	6.0E+04	7.5E+04	4.1E+04	5.3E+04	3.9E+05	4.1E+05
	Stdev	6.5E+03	7.6E+03	3.6E+03	4.8E+03	8.3E+04	1.5E+04
	S:B	**7.8**	**14**	**8.4**	**11**	**1.3**	**1.1**
10 uL	Avg Blank	8.5E+03	4.1E+03	4.9E+03	2.4E+03	5.0E+05	2.5E+05
	Stdev	6.7E+03	2.6E+03	3.3E+03	1.3E+03	7.3E+04	1.7E+04
	Avg Drug	5.6E+04	6.5E+04	4.0E+04	4.7E+04	4.5E+05	3.5E+05
	Stdev	3.1E+03	2.1E+03	1.8E+03	1.7E+03	1.1E+05	5.7E+04
	S:B	**6.6**	**16**	**8.1**	**20**	**0.90**	**1.4**
20 uL	Avg Blank	3.4E+03	8.7E+02	2.0E+03	6.3E+02	2.0E+03	1.2E+05
	Stdev	1.2E+03	4.3E+02	6.2E+02	3.4E+02	6.2E+02	4.6E+04
	Avg Drug	4.6E+04	5.3E+04	3.2E+04	3.7E+04	3.2E+04	2.6E+05
	Stdev	1.4E+04	1.0E+04	9.1E+03	7.2E+03	9.1E+03	7.4E+04
	S:B	**13**	**61**	**16**	**9**	**16**	**2.3**
40 uL	Avg Blank	3.9E+03	8.8E+02	2.6E+03	6.8E+02	3.7E+05	9.8E+04
	Stdev	3.5E+03	1.3E+02	2.2E+03	2.3E+02	7.5E+04	3.3E+04
	Avg Drug	5.4E+04	3.7E+04	3.8E+04	2.6E+04	3.3E+05	2.1E+05
	Stdev	6.5E+03	6.2E+02	4.4E+03	3.2E+02	2.8E+04	5.9E+04
	S:B	**14**	**42**	**15**	**38**	**0.89**	**2.2**

		Zolpidem			
		Whole Blood	Diluted Blood	Whole Blood	Diluted Blood
	Fragment	**308 -> 92**		**308 -> 235**	
5 uL	Avg Blank	2.9E+04	1.7E+04	1.3E+05	7.2E+04
	Stdev	1.3E+04	5.8E+03	6.4E+04	2.9E+04
	Avg Drug	1.7E+05	1.3E+05	8.9E+05	6.7E+05
	Stdev	1.3E+04	1.9E+04	6.7E+04	9.3E+04
	S:B	**6.0**	**8.0**	**6.7**	**9.3**
10 uL	Avg Blank	1.3E+04	8.0E+03	4.4E+04	3.2E+04
	Stdev	9.5E+03	3.6E+03	3.5E+04	1.6E+04
	Avg Drug	2.0E+05	1.4E+05	1.1E+06	7.3E+05
	Stdev	1.3E+04	1.3E+04	8.2E+04	8.1E+04
	S:B	**15**	**17**	**24**	**23**
20 uL	Avg Blank	9.6E+03	2.9E+03	4.2E+04	1.2E+04
	Stdev	3.8E+03	1.2E+03	1.8E+04	5.3E+03
	Avg Drug	1.6E+05	1.0E+05	8.5E+05	5.5E+05
	Stdev	5.4E+04	1.4E+04	2.9E+05	7.3E+04
	S:B	**17**	**36**	**20**	**44**
40 uL	Avg Blank	1.6E+04	4.0E+03	7.9E+04	2.0E+04
	Stdev	1.5E+04	3.7E+02	7.3E+04	4.5E+02
	Avg Drug	1.8E+05	6.7E+04	9.6E+05	3.5E+05
	Stdev	2.0E+04	2.7E+03	1.0E+05	1.3E+04
	S:B	**11**	**17**	**12**	**18**

		Fentanyl					
		Whole Blood	Diluted Blood	Whole Blood	Diluted Blood	Whole Blood	Diluted Blood
	Fragment	**337 -> 79**		**337 -> 105**		**337 -> 188**	
5 uL	Avg Blank	3.4E+04	2.6E+04	4.7E+04	3.4E+04	2.5E+04	1.3E+04
	Stdev	1.7E+04	2.8E+03	2.1E+04	3.0E+03	1.0E+04	5.1E+03
	Avg Drug	7.6E+04	8.1E+04	1.8E+05	1.5E+05	1.5E+05	1.0E+05
	Stdev	1.7E+04	1.5E+04	2.7E+04	1.2E+04	2.0E+04	1.8E+03
	S:B	**2.3**	**3.1**	**3.9**	**4.5**	**6.0**	**7.8**
10 uL	Avg Blank	4.2E+04	1.9E+04	3.9E+04	1.9E+04	7.8E+03	5.2E+03
	Stdev	2.6E+04	7.1E+03	2.4E+04	7.5E+03	5.4E+03	2.3E+03
	Avg Drug	7.5E+04	5.9E+04	2.4E+05	1.4E+05	2.2E+05	1.1E+05
	Stdev	6.1E+03	8.6E+03	2.3E+04	7.0E+03	2.5E+04	1.6E+04
	S:B	**1.8**	**3.2**	**6.2**	**7.6**	**28**	**22**
20 uL	Avg Blank	1.6E+04	4.6E+03	1.6E+04	5.8E+03	6.0E+03	2.8E+03
	Stdev	2.1E+03	2.7E+03	3.5E+03	2.8E+03	2.1E+03	1.3E+03
	Avg Drug	6.4E+04	4.4E+04	6.4E+04	1.3E+05	1.6E+05	1.1E+05
	Stdev	1.8E+04	6.7E+03	1.8E+04	1.3E+04	5.0E+04	1.1E+04
	S:B	**4.0**	**9.4**	**3.9**	**22**	**27**	**38**
40 uL	Avg Blank	1.5E+04	3.6E+03	1.9E+04	6.6E+03	1.1E+04	5.0E+03
	Stdev	1.0E+04	4.8E+02	1.5E+04	5.6E+01	9.0E+03	5.0E+02
	Avg Drug	6.7E+04	2.9E+04	2.4E+05	9.4E+04	2.1E+05	8.7E+04
	Stdev	3.3E+03	2.9E+02	2.1E+04	3.8E+03	2.2E+04	2.2E+03
	S:B	**4.6**	**8.0**	**12**	**14**	**19**	**17**

		Buprenorphine							
		Whole Blood	Diluted Blood	Whole Blood	Diluted Blood	Whole Blood	Diluted Blood	Whole Blood	Diluted Blood
	Fragment	468-> 101		468-> 187		468-> 396		468-> 414	
5 uL	Avg Blank	1.0E+3	8.5E+2	7.0E+3	6.4E+3	9.7E+2	9.1E+2	9.8E+2	9.9E+2
	Stdev	4.2E+2	7.1E+1	1.1E+3	4.3E+2	4.5E+2	8.2E+1	5.2E+2	3.1E+1
	Avg Drug	3.8E+3	4.0E+3	8.9E+3	9.1E+3	6.7E+3	8.1E+3	6.1E+3	6.2E+3
	Stdev	8.2E+2	3.2E+2	7.7E+2	8.9E+2	1.6E+3	1.3E+3	1.2E+3	3.8E+2
	S:B	**3.9**	**4.7**	**1.3**	**1.4**	**6.9**	**8.9**	**6.2**	**6.3**
10 uL	Avg Blank	1.0E+3	6.5E+2	6.1E+3	8.0E+3	1.0E+3	4.5E+2	1.1E+3	3.6E+2
	Stdev	5.2E+02	2.3E+2	1.3E+3	1.1E+3	5.1E+2	1.6E+2	6.5E+2	1.8E+2
	Avg Drug	3.2E+3	3.1E+3	8.3E+3	1.1E+4	6.8E+3	5.0E+3	6.0E+3	4.7E+3
	Stdev	3.2E+2	2.9E+2	9.6E+1	6.1E+2	1.2E+3	2.3E+2	1.1E+3	1.2E+2
	S:B	**3.1**	**4.7**	**1.4**	**1.35**	**6.7**	**11**	**5.5**	**13**
20 uL	Avg Blank	5.5E+2	1.7E+2	5.0E+3	4.5E+3	4.3E+2	1.2E+2	2.1E+2	4.8E+1
	Stdev	1.3E+2	7.9E+1	3.5E+2	7.3E+2	2.2E+1	6.2E+1	5.2E+1	1.9E+1
	Avg Drug	2.5E+3	2.1E+3	7.2E+03	8.9E+3	5.3E+3	4.4E+3	4.6E+3	4.0E+3
	Stdev	9.1E+2	4.9E+2	2.0E+03	7.3E+2	1.7E+3	1.5E+3	1.8E+3	1.2E+3
	S:B	**4.6**	**12**	**1.5**	**1.9**	**12**	**36**	**22**	**84**
40 uL	Avg Blank	4.6E+2	1.8E+2	5.6E+3	5.7E+3	4.3E+2	7.3E+1	3.0E+2	6.4E+1
	Stdev	3.1E+2	4.2E+1	4.3E+2	4.2E+2	3.3E+2	9.4E+0	2.2E+2	1.1E+1
	Avg Drug	2.5E+3	9.0E+2	5.6E+3	5.3E+3	5.5E+3	2.2E+3	5.3E+3	1.9E+3
	Stdev	5.6E+2	2.1E+2	4.0E+2	1.5E+3	1.3E+3	3.6E+2	1.4E+3	4.1E+2
	S:B	**5.4**	**5.0**	**0.99**	**0.95**	**13**	**30**	**17**	**30**

		Clonazepam							
		Whole Blood	Diluted Blood	Whole Blood	Diluted Blood	Whole Blood	Diluted Blood	Whole Blood	Diluted Blood
	Fragment	**316 -> 151**		**316 -> 214**		**316 -> 241**		**316 -> 270**	
5 uL	Avg Blank	7.4E+03	4.8E+03	1.9E+03	2.1E+03	8.9E+02	6.6E+02	1.9E+04	1.6E+04
	Stdev	4.8E+03	9.6E+02	1.3E+03	2.4E+02	6.8E+02	7.8E+01	1.1E+04	9.6E+02
	Avg Drug	1.5E+04	2.2E+04	1.0E+04	1.5E+04	6.5E+03	9.3E+03	4.2E+04	5.7E+04
	Stdev	7.0E+03	6.1E+03	3.4E+03	1.6E+03	2.3E+03	1.2E+03	1.4E+04	1.2E+04
	S:B	**2.0**	**4.6**	**5.4**	**7.1**	**7.3**	**14**	**2.2**	**3.5**
10 uL	Avg Blank	1.2E+04	4.1E+03	2.9E+03	1.0E+03	1.4E+03	4.7E+02	2.5E+04	1.1E+04
	Stdev	9.0E+03	2.6E+03	2.1E+03	5.5E+02	1.0E+03	2.4E+02	1.5E+04	4.3E+03
	Avg Drug	4.9E+03	1.4E+04	4.3E+03	8.1E+03	2.7E+03	5.5E+03	1.8E+04	3.5E+04
	Stdev	1.6E+03	7.2E+03	1.4E+03	3.3E+03	7.8E+02	2.2E+03	4.5E+03	1.4E+04
	S:B	**0.42**	**3.4**	**1.5**	**8.0**	**2.0**	**12**	**0.75**	**3.1**
20 uL	Avg Blank	4.0E+03	1.0E+03	9.2E+02	2.1E+02	3.6E+02	8.7E+01	1.0E+04	3.4E+03
	Stdev	1.1E+03	7.4E+02	3.8E+02	1.2E+02	1.3E+02	5.3E+01	1.9E+03	1.7E+03
	Avg Drug	5.6E+03	4.5E+03	4.7E+03	3.7E+03	2.9E+03	2.4E+03	2.0E+04	1.6E+04
	Stdev	2.6E+03	1.2E+03	1.9E+03	1.2E+03	1.2E+03	7.6E+02	6.5E+03	4.4E+03
	S:B	**1.4**	**4.3**	**5.1**	**18**	**8.1**	**27**	**2.0**	**4.8**
40 uL	Avg Blank	2.3E+03	5.2E+02	1.0E+03	1.5E+02	5.1E+02	3.6E+01	8.0E+03	2.4E+03
	Stdev	1.7E+03	2.0E+02	7.6E+02	6.8E+01	4.5E+02	2.0E+01	4.4E+03	7.3E+02
	Avg Drug	2.7E+03	1.6E+03	2.7E+03	1.2E+03	1.8E+03	6.6E+02	1.1E+04	6.0E+03
	Stdev	8.6E+02	4.0E+02	1.0E+03	2.7E+02	7.0E+02	2.0E+02	2.4E+03	1.5E+03
	S:B	**1.2**	**3.0**	**2.7**	**7.8**	**3.6**	**19**	**1.4**	**2.5**

APPENDIX C

Mass spec parameters for purposed targets after tuning on triple quadrupole

Target	Q1	Q3	CE	S lens	Ion ratio
Acetaminophen	152	110	16	59	100:30
		65	30		
Alfentanil	417	268	17	95	100:62
		197	26		
Alpha-PVP	232	91	24	69	100:41
		77	43		
Alprazolam	309	205	41	111	100:87
		281	25		
Amitriptyline	278	233	16	81	100:61
		117	30		
Amlodipine	409	238	11	58	100:67
		294	11		
Amphetamine	136	91	17	34	100:25
		119	6		
Aripiprazole	448	285	25	146	100:28
		176	31		
Atenolol	267	145	26	85	100:78
		190	18		
Baclofen	214	151	18	52	100:88
		115	45		
Benztropine	308	167	29	106	100:45
		165	50		
Benzoylecgonine	290	168	19	85	100:33
		77	48		
Benzylpiperazine	177	91	22	59	100:12
		85	16		
Brompheniramine	319	274	19	62	100:47
		167	41		
Bupivacaine	289	140	20	73	100:17
		98	34		

Target	Q1	Q3	CE	S lens	Ion ratio
Bupropion	240	184	12	53	100:55
		131	27		
Buspirone	386	122	31	127	100:18
		95	50		
Carbamazepine	237	194	19	82	100:30
		192	23		
Carbamazepine-10,11-epoxide	253	180	30	58	100:36
		210	15		
Carisoprodol	261	97	16	42	100:83
		55	29		
Chlordiazepoxide	300	227	25	75	100:22
		255	20		
Chlorpheniramine	275	230	17	58	100:53
		167	41		
Chlorpromazine	319	214	39	78	100:26
		58	35		
Citalopram	325	262	19	95	100:47
		234	28		
Clomipramine	315	227	40	75	100:73
		242	26		
Clonazepam	316	270	24	136	100:32
		214	36		
Clozapine	327	270	22	88	100:64
		192	43		
Cocaethylene	318	196	19	78	100:27
		82	32		
Cocaine	304	182	19	94	100:21
		82	31		
Codeine	300	215	25	97	100:61
		199	30		
Cyclobenzaprine	276	215	40	87	100:39
		231	17		
Demoxepam	287	180	22	113	100:65
		207	36		

Target	Q1	Q3	CE	S lens	Ion ratio
Desalkylflurazepam	289	140	29	91	100:66
		226	27		
Desipramine	267	72	16	68	100:20
		193	38		
Desmethylclomipramine	301	72	17	73	100:17
		227	37		
Dextromethorphan	272	215	23	107	100:76
		147	30		
Diazepam	285	154	26	94	100:81
		222	26		
Diltiazem	415	178	24	94	100:44
		150	41		
Diphenhydramine	256	167	15	46	100:43
		165	39		
Donepezil	380	91	36	112	100:16
		243	26		
Doxepin	280	107	23	89	100:52
		165	56		
Doxylamine	271	167	34	54	100:88
		182	16		
Duloxetine	298	44	14	41	100:87
		154	5		
EDDP	278	234	31	89	100:42
		249	23		
Ephedrine/Pseudoephedrine	166	115	27	44	100:86
		117	19		
Etomidate	245	141	10	45	100:66
		95	24		
Felbamate	239	117	16	40	100:64
		178	5		
Fentanyl	337	188	22	104	100:70
		105	35		
Flecainide	415	301	32	127	100:38
		98	29		

Target	Q1	Q3	CE	S lens	Ion ratio
Flunitrazepam	314	268	25	91	100:38
		239	33		
Fluoxetine	310	44	14	56	100:13
		148	6		
Flurazepam	388	315	22	90	100:22
		317	18		
Fluvoxamine	319	71	16	67	100:21
		200	21		
Gabapentin	172	137	15	51	100:32
		119	17		
Haloperidol	376	123	37	101	100:93
		165	23		
Hydrocodone	300	199	29	112	100:37
		171	38		
Hydromorphone	286	185	30	97	100:69
		157	40		
Hydroxyzine	375	201	19	71	100:86
		165	61		
Ketamine	238	89	54	62	100:24
		125	30		
Labetalol	329	162	25	71	100:87
		91	36		
Lamotrigine	256	211	26	104	100:63
		145	38		
Levetiracetam	171	126	15	33	100:24
		154	5		
Lidocaine	235	86	18	66	100:12
		58	35		
Lorazepam	321	276	18	65	100:48
		167	41		
mCPP	197	154	19	71	100:89
		118	34		
MDA	180	135	19	39	100:91
		133	18		

Target	Q1	Q3	CE	S lens	Ion ratio
MDMA	194	163	12	48	100:35
		105	24		
MDPV	276	175	22	87	100:89
		135	27		
Meperidine	248	220	21	81	100:58
		174	20		
Mephedrone	178	145	20	54	100:76
		144	31		
Meprobamate	219	158	5	39	100:81
		97	14		
Mescaline	212	180	18	44	100:81
		165	24		
Metaxalone	222	161	11	57	100:26
		105	27		
Methadone	310	265	14	77	100:32
		105	29		
Methamphetamine	150	91	19	50	100:39
		119	11		
Methocarbamol	242	118	11	47	100:23
		199	5		
Methylone	208	160	17	59	100:52
		132	27		
Methylphenidate	234	84	20	69	100:13
		56	41		
Metoclopramide	300	227	18	75	100:39
		184	31		
Metoprolol	268	116	18	83	100:68
		77	52		
Midazolam	326	291	26	98	100:27
		249	37		
Mirtazapine	266	195	26	74	100:41
		194	42		
Morphine	286	152	60	84	100:71
		165	40		

Target	Q1	Q3	CE	S lens	Ion ratio
Naproxen	231	185	14	63	100:30
		170	26		
Norclozapine	313	192	41	90	100:73
		270	24		
Nordiazepam	271	140	28	99	100:68
		208	27		
Nordoxepin	266	107	22	76	100:60
		235	15		
Norfluoxetine	296	134	5	43	100:26
		30	13		
Norketamine	224	125	25	54	100:44
		179	16		
Normeperidine	234	160	16	64	100:22
		56	23		
Norpropoxyphene	308	44	20	60	100:48
		128	43		
Norsertraline	275	159	18	77	100:11
		124	41		
Nortramadol	250	232	5	48	100:25
		42	53		
Nortriptyline	264	233	14	77	100:42
		117	20		
Norvenlafaxine	264	58	19	69	100:24
		107	34		
Olanzapine	313	256	22	96	100:27
		198	41		
Oxazepam	287	241	22	88	100:15
		77	51		
Oxycodone	316	241	28	86	100:63
		212	41		
Oxymorphone	302	227	28	84	100:73
		198	43		
Papaverine	340	324	30	114	100:101
		202	26		

Target	Q1	Q3	CE	S lens	Ion ratio
Paroxetine	330	192	20	88	100:52
		70	30		
PCP	244	91	34	43	100:56
		159	13		
Pentazocine	286	218	20	83	100:22
		69	26		
Phenytoin	253	182	18	63	100:61
		104	34		
Pregabalin	160	97	23	50	100:21
		124	15		
Primidone	219	162	13	51	100:70
		91	32		
Promethazine	285	86	17	60	100:43
		198	27		
Propoxyphene	340	266	5	50	100:20
		128	45		
Propranolol	260	116	18	76	100:88
		183	18		
Quetiapine	384	253	22	97	100:64
		221	36		
Ranitidine	315	176	17	77	100:49
		102	34		
Risperidone	411	191	28	113	100:8
		110	47		
Ropinirole	261	114	19	77	100:21
		132	33		
Sertraline	306	159	29	50	100:85
		275	12		
Sildenafil	475	100	29	158	100:62
		283	38		
Temazepam	301	177	38	87	100:32
		239	45		
TFMPP	231	188	22	76	100:23
		118	39		

Target	Q1	Q3	CE	S lens	Ion ratio
Topiramate	362	265	18	93	100:52
		207	18		
Tramadol	264	264	15	60	100:30
		58	15		
Trazodone	372	176	23	101	100:84
		148	33		
Triazolam	343	308	26	146	100:76
		239	41		
Trimipramine	295	193	41	74	100:71
		208	25		
Vardenafil	489	151	47	152	100:43
		312	37		
Venlafaxine	278	58	18	61	100:25
		121	32		
Verapamil	455	165	28	115	100:36
		150	37		
Zaleplon	306	236	26	97	100:88
		264	22		
Ziprasidone	413	194	27	115	100:37
		159	40		
Zolpidem	308	92	51	103	100:18
		235	33		
Zonisamide	213	132	15	61	100:46
		77	33		
Zopiclone	389	245	16	59	100:39
		112	54		
6-acetylmorphine	328	165	38	100	100:57
		211	25		
7-aminoclonazepam	286	121	30	106	100:36
		94	40		
7-aminoflunitrazepam	284	135	27	100	100:51
		227	25		
9-hydroxyrisperidone	427	207	26	108	100:22
		110	40		

APPENDIX D

Spiking solutions A-C were made by combining stock solutions into 95:5:0.01 methanol:water:acetic acid. Spiking solutions D-J were made by combining stock solutions into 95:5 methanol:water at 200x cutoff and then diluting 1:10 with 95:5:0.01 methanol:water:acetic acid. Solution K was made by combining stock solutions into 95:5 methanol:water at 2000x cutoff and then diluting 1:100 with 95:5:0.01 methanol:water:acetic acid.

Solution A	**Solution B**
Acetaminophen•†	Baclofen
Carisoprodol	Carbamazepine•
Felbamate	Carbamazepine-10,11-epoxide
Levetiracetam•	Lamotrigine
Naproxen†	Meprobamate
Norsertraline†	Metaxalone
Phenytoin†	Methocarbamol•
Primidone	Nortramadol†
Topiramate†	
Tramadol†	
Zonisamide†	

Solution C	Solution D
Amphetamine†	Aripiprazole•†
Benzylpiperazine†	Desmethylclomipramine†
Bupivacaine•	Etomidate•
Flecainide	Ketamine
Gabapentin	MDA†
Lidocaine	Mescaline†
Papaverine•	Metoclopramide•
Pregabalin	Norketamine
Ranitidine•	Oxycodone†
	Sertraline
	Sildenafil
	Trazodone
	Vardenafil
	Ziprasidone†
Solution E	**Solution F**
Demoxepam†	Donepezil•
Ephedrine/Pseudoephedrine	Labetalol•
Hydroxyzine•†	MDMA
Norpropoxyphene	MDPV
Norvenlafaxine	Mephedrone
Olanzapine	Methamphetamine
Pentazocine	Methylone
Propoxyphene	Metoprolol
Propranolol	Midazolam
Quetiapine	Mirtazapine
TFMPP	Norclozapine
Venlafaxine	
Verapamil	

Solution G	**Solution H**
6-acetylmorphine†	Brompheniramine
7-aminoclonazepam†	Diphenhydramine
Alfentanil	Doxylamine
Alpha-PVP	EDDP
Bupropion	Flurazepam
Chlordiazepoxide	Lorazepam
Chlorpromazine	Meperidine
Clozapine	Normeperidine
Cocaethylene	PCP
Desalkylflurazepam	Promethazine
Diltiazem	
Solution I	**Solution J**
Buspirone†	7-aminoflunitrazepam
Chlorpheniramine	Amlodipine•
Clonazepam†	Benztropine•
Fluvoxamine•†	Clomipramine
Methadone	Desipramine
Morphine†	Duloxetine
Oxymorphone†	Flunitrazepam
Paroxetine†	Fluoxetine
Zaleplon	mCPP
Zopiclone†	Methylphenidate
	Nordoxepin
	Norfluoxetine
	Triazolam
	Trimipramine

Solution K	Solution L
9-hydroxyrisperidone Alprazolam Cyclobenzaprine Dextromethorphan Fentanyl† Haloperidol Risperidone Ropinirole• Zolpidem	Amitriptyline Atenolol Benzoylecgonine Diazepam Hydrocodone Nortriptyline
Solution M	
Citalopram Cocaine Codeine Doxepine Hydromorphone Nordiazepam Oxazepam Temazepam	

- Original solutions made by dissolving powdered Sigma reagents

† Target run at a concentration other than that specified by AFT

APPENDIX E

Detailed data by cocktail

Acetaminophen				
Cocktail A				
		m/z 110	**m/z 65**	**n**
Manual	Spiked Average	1.02E+07	2.87E+06	4
	Spiked RSD	19%	19%	
	Blank Average	3.49E+05	1.16E+05	4
	Blank RSD	173%	144%	
Laser-cut	Spiked Average	1.75E+07	4.96E+06	4
	Spiked RSD	49%	51%	
	Blank Average	1.26E+05	8.96E+04	4
	Blank RSD	29%	27%	
Levetiracetam				
Cocktail A				
		m/z 126	**m/z 154**	**n**
Manual	Spiked Average	9.37E+05	2.40E+05	4
	Spiked RSD	10%	9%	
	Blank Average	4.01E+04	1.21E+04	4
	Blank RSD	167%	143%	
Laser-cut	Spiked Average	3.78E+06	9.94E+05	4
	Spiked RSD	43%	47%	
	Blank Average	7.95E+04	1.17E+04	4
	Blank RSD	42%	37%	

Zonisamide				
Cocktail A				
		m/z 132	m/z 77	n
Manual	Spiked Average	4.54E+06	2.19E+06	4
	Spiked RSD	23%	23%	
	Blank Average	1.30E+05	1.11E+05	4
	Blank RSD	172%	110%	
Laser-cut	Spiked Average	6.52E+06	3.08E+06	4
	Spiked RSD	59%	59%	
	Blank Average	9.71E+03	9.83E+04	4
	Blank RSD	33%	22%	
Primidone				
Cocktail A				
		m/z 162	m/z 91	n
Manual	Spiked Average	2.55E+06	1.83E+06	4
	Spiked RSD	33%	32%	
	Blank Average	6.32E+04	1.20E+05	4
	Blank RSD	172%	86%	
Laser-cut	Spiked Average	3.48E+06	4.09E+06	4
	Spiked RSD	56%	44%	
	Blank Average	1.19E+04	2.27E+06	4
	Blank RSD	35%	19%	
Naproxen				
Cocktail A				
		m/z 185	m/z 170	n
Manual	Spiked Average	1.12E+06	3.09E+05	4
	Spiked RSD	15%	17%	
	Blank Average	1.66E+05	1.50E+04	4
	Blank RSD	58%	105%	
Laser-cut	Spiked Average	3.48E+06	5.42E+05	4
	Spiked RSD	56%	56%	
	Blank Average	1.19E+04	7.32E+04	4
	Blank RSD	35%	21%	

Felbamate				
Cocktail A				
		m/z 117	m/z 178	n
Manual	Spiked Average	7.17E+05	4.37E+05	4
	Spiked RSD	22%	23%	
	Blank Average	5.26E+04	1.17E+04	4
	Blank RSD	76%	173%	
Laser-cut	Spiked Average	1.68E+06	1.07E+06	4
	Spiked RSD	48%	48%	
	Blank Average	3.24E+04	1.35E+03	4
	Blank RSD	18%	35%	
Phenytoin				
Cocktail A				
		m/z 182	m/z 104	n
Manual	Spiked Average	5.49E+05	3.60E+05	4
	Spiked RSD	80%	80%	
	Blank Average	1.29E+04	8.19E+03	4
	Blank RSD	121%	95%	
Laser-cut	Spiked Average	2.75E+05	2.10E+05	4
	Spiked RSD	67%	69%	
	Blank Average	2.25E+04	3.61E+04	4
	Blank RSD	28%	34%	
Carisoprodol				
Cocktail A				
		m/z 97	m/z 55	n
Manual	Spiked Average	5.46E+05	3.75E+05	4
	Spiked RSD	37%	29%	
	Blank Average	1.42E+05	8.43E+04	4
	Blank RSD	33%	45%	
Laser-cut	Spiked Average	1.59E+06	1.25E+06	4
	Spiked RSD	31%	34%	
	Blank Average	8.38E+04	4.26E+04	4
	Blank RSD	22%	22%	

Tramadol				
Cocktail A				
		m/z 264	m/z 58	n
Manual	Spiked Average	1.58E+07	4.50E+07	4
	Spiked RSD	46%	48%	
	Blank Average	1.55E+06	1.16E+06	4
	Blank RSD	52%	178%	
Laser-cut	Spiked Average	7.75E+07	1.36E+08	4
	Spiked RSD	18%	17%	
	Blank Average	3.40E+07	2.64E+04	4
	Blank RSD	21%	25%	
Norsertraline				
Cocktail A				
		m/z 158	m/z 123	n
Manual	Spiked Average	3.43E+06	2.40E+05	4
	Spiked RSD	41%	43%	
	Blank Average	1.17E+05	6.87E+03	4
	Blank RSD	81%	93%	
Laser-cut	Spiked Average	1.12E+07	7.89E+05	4
	Spiked RSD	11%	12%	
	Blank Average	1.68E+05	1.38E+04	4
	Blank RSD	19%	37%	

Meprobamate				
Cocktail B				
		m/z 158	m/z 97	n
Manual	Spiked Average	5.77E+05	5.09E+05	4
	Spiked RSD	20%	20%	
	Blank Average	2.78E+03	2.05E+04	4
	Blank RSD	51%	30%	
Laser-cut	Spiked Average	9.12E+05	7.95E+05	4
	Spiked RSD	21%	21%	
	Blank Average	1.84E+03	3.28E+04	4
	Blank RSD	31%	18%	
Metaxalone				
Cocktail B				
		m/z 161	m/z 105	n
Manual	Spiked Average	1.42E+06	3.83E+05	4
	Spiked RSD	21%	21%	
	Blank Average	1.20E+04	1.58E+04	4
	Blank RSD	29%	32%	
Laser-cut	Spiked Average	1.77E+06	5.04E+05	4
	Spiked RSD	19%	19%	
	Blank Average	1.57E+04	5.78E+04	4
	Blank RSD	27%	25%	
Carbamazepine				
Cocktail B				
		m/z 194	m/z 192	n
Manual	Spiked Average	1.09E+07	2.45E+06	4
	Spiked RSD	14%	14%	
	Blank Average	6.04E+04	1.32E+04	4
	Blank RSD	49%	47%	
Laser-cut	Spiked Average	2.57E+07	5.76E+06	4
	Spiked RSD	25%	24%	
	Blank Average	2.90E+04	2.76E+04	4
	Blank RSD	35%	38%	

Methocarbamol				
Cocktail B				
		m/z 118	m/z 199	n
Manual	Spiked Average	3.49E+05	7.21E+04	4
	Spiked RSD	23%	26%	
	Blank Average	3.24E+03	1.12E+03	4
	Blank RSD	30%	29%	
Laser-cut	Spiked Average	6.46E+05	1.33E+05	4
	Spiked RSD	24%	24%	
	Blank Average	5.92E+03	1.41E+03	4
	Blank RSD	27%	26%	
Nortramadol				
Cocktail B				
		m/z 232	m/z 42	n
Manual	Spiked Average	4.78E+05	9.98E+04	4
	Spiked RSD	11%	20%	
	Blank Average	9.97E+04	2.96E+03	4
	Blank RSD	14%	23%	
Laser-cut	Spiked Average	1.29E+06	2.98E+05	4
	Spiked RSD	16%	16%	
	Blank Average	2.59E+05	1.44E+04	4
	Blank RSD	30%	32%	
Carbamazepine-10,11-epoxide				
Cocktail B				
		m/z 180	m/z 210	n
Manual	Spiked Average	3.58E+06	1.39E+06	4
	Spiked RSD	17%	17%	
	Blank Average	1.45E+04	1.53E+04	4
	Blank RSD	51%	27%	
Laser-cut	Spiked Average	1.01E+07	3.86E+06	4
	Spiked RSD	26%	26%	
	Blank Average	3.46E+04	2.15E+04	4
	Blank RSD	20%	28%	

Lamotrigine				
Cocktail B				
		m/z 210	m/z 144	n
Manual	Spiked Average	2.97E+06	1.85E+06	4
	Spiked RSD	16%	16%	
	Blank Average	3.52E+04	2.39E+04	4
	Blank RSD	66%	61%	
Laser-cut	Spiked Average	4.53E+06	2.88E+06	4
	Spiked RSD	16%	16%	
	Blank Average	2.50E+04	3.47E+04	4
	Blank RSD	24%	21%	
Baclofen				
Cocktail B				
		m/z 151	m/z 115	n
Manual	Spiked Average	2.82E+06	2.37E+06	4
	Spiked RSD	11%	10%	
	Blank Average	2.99E+04	3.06E+04	4
	Blank RSD	48%	34%	
Laser-cut	Spiked Average	7.84E+05	7.02E+05	4
	Spiked RSD	32%	31%	
	Blank Average	2.24E+04	6.51E+04	4
	Blank RSD	39%	20%	

Amphetamine				
Cocktail C				
		m/z 91	m/z 119	n
Manual	Spiked Average	2.83E+06	6.61E+05	4
	Spiked RSD	13%	16%	
	Blank Average	3.17E+05	2.99E+04	4
	Blank RSD	17%	23%	
Laser-cut	Spiked Average	4.53E+06	9.61E+05	4
	Spiked RSD	16%	14%	
	Blank Average	7.48E+05	2.05E+04	4
	Blank RSD	22%	31%	
Pregabalin				
Cocktail C				
		m/z 97	m/z 124	n
Manual	Spiked Average	1.26E+05	5.88E+05	4
	Spiked RSD	9%	10%	
	Blank Average	4.48E+03	8.69E+03	4
	Blank RSD	24%	36%	
Laser-cut	Spiked Average	1.11E+05	4.98E+05	4
	Spiked RSD	27%	27%	
	Blank Average	1.70E+04	4.26E+04	4
Flecainide				
Cocktail C				
		m/z 301	m/z 98	n
Manual	Spiked Average	6.75E+06	2.73E+06	4
	Spiked RSD	23%	24%	
	Blank Average	2.17E+04	8.15E+03	4
	Blank RSD	78%	86%	
Laser-cut	Spiked Average	3.47E+07	1.39E+07	4
	Spiked RSD	19%	18%	
	Blank Average	1.71E+04	6.47E+03	4
	Blank RSD	15%	15%	

Gabapentin				
Cocktail C				
		m/z 137	m/z 119	n
Manual	Spiked Average	1.80E+06	5.70E+05	4
	Spiked RSD	9%	9%	
	Blank Average	1.55E+04	5.54E+03	4
	Blank RSD	36%	27%	
Laser-cut	Spiked Average	1.78E+06	1.45E+07	4
	Spiked RSD	30%	9%	
	Blank Average	1.21E+04	4.30E+05	4
	Blank RSD	44%	19%	
Benzylpiperazine				
Cocktail C				
		m/z 91	m/z 85	n
Manual	Spiked Average	4.92E+06	1.03E+06	4
	Spiked RSD	18%	9%	
	Blank Average	3.16E+05	3.00E+05	4
	Blank RSD	39%	19%	
Laser-cut	Spiked Average	1.45E+07	1.73E+06	4
	Spiked RSD	9%	9%	
	Blank Average	4.30E+05	4.21E+04	4
	Blank RSD	19%	42%	
Lidocaine				
Cocktail C				
		m/z 86	m/z 58	n
Manual	Spiked Average	2.19E+07	2.22E+06	4
	Spiked RSD	13%	13%	
	Blank Average	5.35E+04	7.14E+03	4
	Blank RSD	118%	101%	
Laser-cut	Spiked Average	6.09E+07	6.14E+06	4
	Spiked RSD	13%	14%	
	Blank Average	8.59E+03	1.04E+05	4
	Blank RSD	22%	25%	

Bupivacaine				
Cocktail C				
		m/z 140	m/z 98	n
Manual	Spiked Average	4.26E+07	7.09E+06	4
	Spiked RSD	24%	24%	
	Blank Average	1.27E+05	2.50E+04	4
	Blank RSD	101%	87%	
Laser-cut	Spiked Average	1.48E+08	2.47E+07	4
	Spiked RSD	17%	17%	
	Blank Average	1.06E+04	1.16E+04	4
	Blank RSD	25%	33%	
Ranitidine				
Cocktail C				
		m/z 176	m/z 102	n
Manual	Spiked Average	7.79E+06	3.96E+06	4
	Spiked RSD	28%	28%	
	Blank Average	3.46E+04	8.87E+03	4
	Blank RSD	47%	97%	
Laser-cut	Spiked Average	3.92E+07	1.99E+07	4
	Spiked RSD	33%	35%	
	Blank Average	5.50E+04	1.93E+03	4
	Blank RSD	7%	55%	
Papaverine				
Cocktail C				
		m/z 324	m/z 202	n
Manual	Spiked Average	2.55E+07	2.67E+07	4
	Spiked RSD	34%	33%	
	Blank Average	7.35E+04	7.81E+04	4
	Blank RSD	99%	98%	
Laser-cut	Spiked Average			4
	Spiked RSD			
	Blank Average			4
	Blank RSD			

Mescaline				
Cocktail D				
		m/z 180	m/z 165	n
Manual	Spiked Average	3.42E+05	2.19E+05	4
	Spiked RSD	30%	43%	
	Blank Average	5.28E+04	4.59E+03	4
	Blank RSD	28%	51%	
Laser-cut	Spiked Average	9.03E+05	2.74E+05	3
	Spiked RSD	10%	8%	
	Blank Average	5.71E+05	3.98E+04	2
	Blank RSD	17%	32%	
Die-cut	Spiked	3.70E+05	2.30E+05	1
	Spiked RSD	-	-	
	Blank Average	9.96E+04	4.18E+03	3
	Blank RSD	32%	57%	
Norketamine				
Cocktail D				
		m/z 125	m/z 179	n
Manual	Spiked Average	1.36E+06	6.12E+05	4
	Spiked RSD	41%	40%	
	Blank Average	1.27E+04	9.18E+03	4
	Blank RSD	79%	56%	
Laser-cut	Spiked Average	2.32E+06	1.07E+06	3
	Spiked RSD	13%	15%	
	Blank Average	1.97E+04	3.37E+04	2
	Blank RSD	11%	5%	
Die-cut	Spiked	2.96E+06	1.33E+06	1
	Spiked RSD	-	-	
	Blank Average	5.46E+03	8.34E+03	3
	Blank RSD	47%	42%	

Ketamine				
Cocktail D				
		m/z 89	**m/z** 125	**n**
Manual	Spiked Average	4.91E+05	1.99E+06	4
	Spiked RSD	31%	30%	
	Blank Average	5.42E+03	1.41E+04	4
	Blank RSD	77%	104%	
Laser-cut	Spiked Average	1.22E+06	4.72E+06	3
	Spiked RSD	23%	21%	
	Blank Average	3.89E+04	3.00E+04	2
	Blank RSD	14%	15%	
Die-cut	Spiked	1.84E+06	7.34E+06	1
	Spiked RSD	-	-	
	Blank Average	8.81E+03	4.75E+03	3
	Blank RSD	71%	51%	
Etomidate				
Cocktail D				
		m/z 141	m/z 95	n
Manual	Spiked Average	2.15E+06	2.27E+06	4
	Spiked RSD	29%	9%	
	Blank Average	1.08E+05	4.46E+05	4
	Blank RSD	49%	67%	
Laser-cut	Spiked Average	-	2.47E+06	3
	Spiked RSD	-	29%	
	Blank Average	-	7.45E+04	2
	Blank RSD	-	10%	
Die-cut	Spiked Average	-	2.52E+06	1
	Spiked RSD	-	-	
	Blank Average	-	8.75E+04	3
	Blank RSD	-	47%	

Metoclopramide				
Cocktail D				
		m/z 227	m/z 184	n
Manual	Spiked Average	7.39E+06	2.75E+06	4
	Spiked RSD	34%	34%	
	Blank Average	6.70E+04	3.10E+04	4
	Blank RSD	107%	77%	
Laser-cut	Spiked Average	3.02E+07	1.14E+07	3
	Spiked RSD	32%	31%	
	Blank Average	1.90E+04	4.57E+04	2
	Blank RSD	16%	6%	
Die-cut	Spiked	7.51E+07	2.96E+07	1
	Spiked RSD	-	-	
	Blank Average	1.16E+04	2.04E+04	3
	Blank RSD	60%	65%	
Desmethylclomipramine				
Cocktail D				
		m/z 72	m/z 227	n
Manual	Spiked Average	3.77E+05	9.10E+04	4
	Spiked RSD	36%	28%	
	Blank Average	3.49E+03	8.18E+03	4
	Blank RSD	68%	32%	
Laser-cut	Spiked Average	1.52E+06	3.55E+05	3
	Spiked RSD	6%	13%	
	Blank Average	1.23E+03	2.77E+04	2
	Blank RSD	6%	11%	
Die-cut	Spiked	1.98E+06	5.09E+05	1
	Spiked RSD	-	-	
	Blank Average	1.15E+03	1.70E+04	3
	Blank RSD	83%	78%	

Sertraline				
Cocktail D				
		m/z 158	m/z 275	n
Manual	Spiked Average	2.66E+05	2.16E+05	4
	Spiked RSD	33%	37%	
	Blank Average	8.49E+03	2.66E+03	4
	Blank RSD	44%	65%	
Laser-cut	Spiked Average	8.95E+05	7.37E+05	3
	Spiked RSD	6%	6%	
	Blank Average	1.89E+04	2.11E+03	2
	Blank RSD	4%	16%	
Die-cut	Spiked	9.17E+05	7.68E+05	1
	Spiked RSD	-	-	
	Blank Average	1.27E+04	2.19E+03	3
MDA				
Cocktail D				
		m/z 135	m/z 133	n
Manual	Spiked Average	2.86E+05	2.66E+05	4
	Spiked RSD	33%	34%	
	Blank Average	1.15E+04	1.54E+04	4
	Blank RSD	38%	28%	
Laser-cut	Spiked Average	4.48E+05	4.48E+05	3
	Spiked RSD	5%	5%	
	Blank Average	3.78E+04	3.78E+04	2
	Blank RSD	22%	22%	
Die-cut	Spiked	4.87E+05	4.87E+05	1
	Spiked RSD	-	-	
	Blank Average	2.08E+04	2.08E+04	3
	Blank RSD	46%	46%	

Oxycodone				
Cocktail D				
		m/z 241	m/z 212	n
Manual	Spiked Average	2.84E+05	1.84E+05	4
	Spiked RSD	36%	33%	
	Blank Average	9.80E+03	9.51E+03	4
	Blank RSD	36%	39%	
Laser-cut	Spiked Average	4.93E+05	3.42E+05	3
	Spiked RSD	17%	19%	
	Blank Average	1.51E+04	3.31E+04	2
	Blank RSD	19%	13%	
Die-cut	Spiked	4.52E+05	3.06E+05	1
	Spiked RSD	-	-	
	Blank Average	7.39E+03	1.24E+04	3
	Blank RSD	66%	70%	
Trazodone				
Cocktail D				
		m/z 176	m/z 148	n
Manual	Spiked Average	2.52E+06	2.09E+06	4
	Spiked RSD	36%	35%	
	Blank Average	3.54E+04	2.76E+04	4
	Blank RSD	52%	53%	
Laser-cut	Spiked Average	9.91E+06	8.19E+06	3
	Spiked RSD	23%	24%	
	Blank Average	2.29E+04	4.90E+04	2
	Blank RSD	6%	7%	
Die-cut	Spiked	1.33E+07	1.10E+07	1
	Spiked RSD	-	-	
	Blank Average	4.19E+04	1.20E+04	3
	Blank RSD	35%	62%	

Ziprasidone				
Cocktail D				
		m/z 194	m/z 159	n
Manual	Spiked Average	7.30E+05	5.93E+05	4
	Spiked RSD	49%	23%	
	Blank Average	5.68E+03	9.76E+04	4
	Blank RSD	69%	68%	
Laser-cut	Spiked Average	1.32E+06	5.98E+05	3
	Spiked RSD	16%	16%	
	Blank Average	1.50E+04	9.83E+04	2
	Blank RSD	6%	0%	
Die-cut	Spiked	7.82E+05	3.43E+05	1
	Spiked RSD	-	-	
	Blank Average	6.23E+03	4.46E+04	3
	Blank RSD	54%	34%	
Aripiprazole				
Cocktail D				
		m/z 285	m/z 176	n
Manual	Spiked Average	8.62E+05	2.46E+05	4
	Spiked RSD	46%	43%	
	Blank Average	1.16E+04	7.07E+03	4
	Blank RSD	48%	27%	
Laser-cut	Spiked Average	1.93E+06	5.46E+05	3
	Spiked RSD	11%	11%	
	Blank Average	8.94E+03	9.71E+03	2
	Blank RSD	4%	20%	
Die-cut	Spiked	1.50E+06	4.28E+05	1
	Spiked RSD	-	-	
	Blank Average	3.85E+03	9.49E+03	3
	Blank RSD	50%	52%	

Sildenafil				
Cocktail D				
		m/z 100	m/z 283	n
Manual	Spiked Average	2.16E+05	1.50E+05	4
	Spiked RSD	42%	35%	
	Blank Average	1.51E+03	6.02E+03	4
	Blank RSD	92%	36%	
Laser-cut	Spiked Average	2.09E+05	1.41E+05	3
	Spiked RSD	22%	24%	
	Blank Average	4.20E+02	7.15E+03	2
	Blank RSD	3%	3%	
Die-cut	Spiked	1.63E+05	1.07E+05	1
	Spiked RSD	-	-	
	Blank Average	3.47E+02	2.29E+03	3
	Blank RSD	97%	74%	
Vardenafil				
Cocktail D				
		m/z 151	m/z 312	n
Manual	Spiked Average	5.39E+05	2.14E+05	4
	Spiked RSD	40%	43%	
	Blank Average	1.40E+04	2.17E+03	4
	Blank RSD	36%	90%	
Laser-cut	Spiked Average	1.20E+06	4.88E+05	3
	Spiked RSD	28%	26%	
	Blank Average	5.76E+03	2.08E+03	2
	Blank RSD	4%	9%	
Die-cut	Spiked Average	9.63E+05	4.05E+05	1
	Spiked RSD	-	-	
	Blank Average	3.59E+03	8.53E+02	3
	Blank RSD	66%	70%	

Ephedrine/Pseudoephedrine				
Cocktail E				
		m/z 115	m/z 117	n
Manual	Spiked Average	1.85E+05	1.54E+05	4
	Spiked RSD	4%	6%	
	Blank Average	7.04E+03	5.66E+03	4
	Blank RSD	112%	108%	
Laser-cut	Spiked Average	3.55E+05	3.07E+05	4
	Spiked RSD	36%	37%	
	Blank Average	3.87E+03	9.06E+03	2
	Blank RSD	1%	28%	
Die-cut	Spiked Average	4.39E+05	3.62E+05	4
	Spiked RSD	13%	13%	
	Blank Average	9.95E+03	7.31E+03	3
	Blank RSD	41%	56%	
TFMPP				
Cocktail E				
		m/z 188	m/z 118	n
Manual	Spiked Average	6.26E+05	1.50E+05	4
	Spiked RSD	11%	10%	
	Blank Average	1.49E+04	6.71E+03	4
	Blank RSD	96%	66%	
Laser-cut	Spiked Average	7.98E+05	2.45E+05	4
	Spiked RSD	11%	25%	
	Blank Average	3.16E+04	4.23E+04	2
	Blank RSD	7%	10%	
Die-cut	Spiked Average	1.20E+06	2.85E+05	4
	Spiked RSD	15%	17%	
	Blank Average	2.19E+04	1.42E+04	3
	Blank RSD	63%	63%	

Propranolol				
Cocktail E				
		m/z 116	m/z 183	n
Manual	Spiked Average	3.13E+05	2.80E+05	4
	Spiked RSD	8%	8%	
	Blank Average	7.13E+03	1.23E+04	4
	Blank RSD	125%	73%	
Laser-cut	Spiked Average	8.76E+05	7.77E+05	4
	Spiked RSD	21%	22%	
	Blank Average	6.21E+03	1.49E+04	2
	Blank RSD	15%	1%	
Die-cut	Spiked Average	1.04E+06	9.41E+05	4
	Spiked RSD	12%	11%	
	Blank Average	1.86E+03	1.31E+04	3
	Blank RSD	67%	36%	
Norvenlafaxine				
Cocktail E				
		m/z 58	m/z 107	n
Manual	Spiked Average	5.46E+05	1.30E+05	4
	Spiked RSD	12%	12%	
	Blank Average	3.26E+04	1.04E+04	4
	Blank RSD	123%	52%	
Laser-cut	Spiked Average	1.95E+06	6.53E+05	4
	Spiked RSD	53%	57%	
	Blank Average	3.13E+04	1.94E+05	2
	Blank RSD	41%	19%	
Die-cut	Spiked Average	2.39E+06	6.22E+05	4
	Spiked RSD	21%	22%	
	Blank Average	4.90E+03	9.72E+04	3
	Blank RSD	39%	68%	

Venlafaxine				
Cocktail E				
		m/z 58	m/z 121	n
Manual	Spiked Average	9.77E+05	2.56E+05	4
	Spiked RSD	17%	17%	
	Blank Average	1.05E+04	1.26E+04	4
	Blank RSD	150%	56%	
Laser-cut	Spiked Average	3.70E+06	1.34E+06	4
	Spiked RSD	47%	46%	
	Blank Average	1.61E+04	1.74E+05	2
	Blank RSD	21%	9%	
Die-cut	Spiked Average	5.80E+06	1.52E+06	4
	Spiked RSD	24%	24%	
	Blank Average	6.48E+03	9.21E+04	3
	Blank RSD	75%	68%	
Pentazocine				
Cocktail E				
		m/z 218	m/z 69	n
Manual	Spiked Average	1.58E+06	3.75E+05	4
	Spiked RSD	15%	14%	
	Blank Average	2.18E+04	3.47E+04	4
	Blank RSD	132%	40%	
Laser-cut	Spiked Average	6.86E+06	1.60E+06	4
	Spiked RSD	40%	40%	
	Blank Average	2.55E+04	1.06E+05	2
	Blank RSD	7%	11%	
Die-cut	Spiked Average	9.67E+06	2.17E+06	4
	Spiked RSD	25%	25%	
	Blank Average	1.25E+04	1.26E+05	3
	Blank RSD	52%	70%	

Demoxepam				
Cocktail E				
		m/z 179	m/z 207	n
Manual	Spiked Average	1.91E+04	1.52E+04	4
	Spiked RSD	21%	18%	
	Blank Average	3.17E+03	5.62E+03	4
	Blank RSD	76%	68%	
Laser-cut	Spiked Average	4.53E+04	3.14E+04	4
	Spiked RSD	56%	59%	
	Blank Average	7.20E+03	6.92E+03	2
	Blank RSD	16%	7%	
Die-cut	Spiked Average	4.43E+04	2.95E+04	4
	Spiked RSD	41%	45%	
	Blank Average	2.40E+03	2.93E+03	3
	Blank RSD	62%	64%	
Norpropoxyphene				
Cocktail E				
		m/z 44	m/z 128	n
Manual	Spiked Average	2.62E+05	1.28E+05	4
	Spiked RSD	20%	19%	
	Blank Average	4.31E+03	6.32E+03	4
	Blank RSD	115%	72%	
Laser-cut	Spiked Average	1.39E+06	7.25E+05	4
	Spiked RSD	27%	22%	
	Blank Average	2.32E+03	2.94E+04	2
	Blank RSD	17%	8%	
Die-cut	Spiked Average	1.98E+06	9.67E+05	4
	Spiked RSD	31%	32%	
	Blank Average	2.32E+03	1.37E+04	3
	Blank RSD	52%	55%	

Olanzapine				
Cocktail E				
		m/z 256	m/z 198	n
Manual	Spiked Average	1.12E+05	3.07E+04	4
	Spiked RSD	16%	14%	
	Blank Average	4.02E+03	2.78E+03	4
	Blank RSD	66%	52%	
Laser-cut	Spiked Average	5.66E+05	1.74E+05	4
	Spiked RSD	32%	41%	
	Blank Average	8.51E+03	2.20E+04	2
	Blank RSD	19%	3%	
Die-cut	Spiked Average	4.10E+05	1.35E+05	4
	Spiked RSD	22%	29%	
	Blank Average	1.05E+04	7.75E+04	3
	Blank RSD	57%	83%	
Propoxyphene				
Cocktail E				
		m/z 266	m/z 128	n
Manual	Spiked Average	3.23E+05	6.67E+04	4
	Spiked RSD	14%	13%	
	Blank Average	1.03E+04	6.59E+03	4
	Blank RSD	64%	70%	
Laser-cut	Spiked Average	1.26E+06	2.68E+05	4
	Spiked RSD	48%	47%	
	Blank Average	3.76E+03	1.89E+04	2
	Blank RSD	5%	1%	
Die-cut	Spiked Average	1.73E+06	3.43E+05	4
	Spiked RSD	25%	25%	
	Blank Average	4.07E+03	1.59E+04	3
	Blank RSD	15%	75%	

Hydroxyzine				
Cocktail E				
		m/z 201	m/z 165	n
Manual	Spiked Average	2.20E+06	1.82E+06	4
	Spiked RSD	10%	10%	
	Blank Average	7.89E+04	3.24E+04	4
	Blank RSD	63%	109%	
Laser-cut	Spiked Average	8.73E+06	7.46E+06	4
	Spiked RSD	40%	42%	
	Blank Average	4.40E+04	7.12E+03	2
	Blank RSD	14%	4%	
Die-cut	Spiked Average	1.19E+07	1.00E+07	4
	Spiked RSD	20%	20%	
	Blank Average	9.63E+04	5.27E+03	3
	Blank RSD	48%	65%	
Quetiapine				
Cocktail E				
		m/z 253	m/z 221	n
Manual	Spiked Average	2.36E+06	1.51E+06	4
	Spiked RSD	11%	10%	
	Blank Average	3.58E+04	1.87E+04	4
	Blank RSD	109%	128%	
Laser-cut	Spiked Average	9.93E+06	6.44E+06	4
	Spiked RSD	52%	53%	
	Blank Average	9.03E+03	1.34E+04	2
	Blank RSD	13%	5%	
Die-cut	Spiked Average	1.08E+07	6.97E+06	4
	Spiked RSD	25%	25%	
	Blank Average	9.96E+03	8.58E+03	3
	Blank RSD	83%	84%	

Verapamil				
Cocktail E				
		m/z 165	m/z	n
Manual	Spiked Average	1.57E+06	5.76E+05	4
	Spiked RSD	10%	10%	
	Blank Average	4.59E+04	7.21E+03	4
	Blank RSD	72%	138%	
Laser-cut	Spiked Average	8.45E+06	3.16E+06	4
	Spiked RSD	14%	14%	
	Blank Average	1.72E+04	8.86E+03	2
	Blank RSD	6%	28%	
Die-cut	Spiked Average	1.16E+07	4.30E+06	4
	Spiked RSD	26%	26%	
	Blank Average	1.80E+04	2.94E+03	3
	Blank RSD	44%	54%	

Mephedrone				
Cocktail F				
		m/z 145	m/z 144	n
Manual	Spiked Average	1.09E+06	8.11E+05	4
	Spiked RSD	8%	9%	
	Blank Average	1.39E+04	7.25E+03	4
	Blank RSD	42%	77%	
Laser-cut	Spiked Average	1.85E+06	1.39E+06	4
	Spiked RSD	20%	19%	
	Blank Average	1.40E+04	1.22E+04	2
	Blank RSD	25%	44%	
Die-cut	Spiked Average	4.78E+06	3.58E+06	4
	Spiked RSD	3%	4%	
	Blank Average	2.08E+04	1.28E+04	3
	Blank RSD	61%	34%	
MDMA				
Cocktail F				
		m/z 163	m/z 105	n
Manual	Spiked Average	1.11E+06	4.03E+05	4
	Spiked RSD	7%	7%	
	Blank Average	9.74E+03	1.01E+04	4
	Blank RSD	68%	42%	
Laser-cut	Spiked Average	2.26E+06	8.64E+05	4
	Spiked RSD	17%	19%	
	Blank Average	5.76E+03	4.23E+04	2
	Blank RSD	31%	30%	
Die-cut	Spiked Average	3.58E+06	1.32E+06	4
	Spiked RSD	10%	11%	
	Blank Average	5.30E+03	5.83E+04	3
	Blank RSD	95%	80%	

Methylone				
Cocktail F				
		m/z 160	m/z 132	n
Manual	Spiked Average	1.09E+06	5.76E+05	4
	Spiked RSD	8%	9%	
	Blank Average	1.88E+04	1.59E+04	4
	Blank RSD	43%	18%	
Laser-cut	Spiked Average	2.08E+06	1.13E+06	4
	Spiked RSD	23%	26%	
	Blank Average	6.75E+04	7.23E+04	2
	Blank RSD	7%	13%	
Die-cut	Spiked Average	4.00E+06	2.10E+06	4
	Spiked RSD	13%	13%	
	Blank Average	1.85E+04	4.70E+04	3
	Blank RSD	112%	80%	
Mirtazapine				
Cocktail F				
		m/z 195	m/z 194	n
Manual	Spiked Average	1.60E+06	6.46E+05	4
	Spiked RSD	9%	9%	
	Blank Average	1.47E+04	6.39E+03	4
	Blank RSD	57%	43%	
Laser-cut	Spiked Average	3.77E+06	1.57E+06	4
	Spiked RSD	26%	27%	
	Blank Average	2.25E+04	6.16E+04	2
	Blank RSD	1%	19%	
Die-cut	Spiked Average	6.69E+06	2.73E+06	4
	Spiked RSD	7%	7%	
	Blank Average	2.03E+04	5.24E+04	3
	Blank RSD	49%	46%	

Metoprolol				
Cocktail F				
		m/z 116	m/z 77	n
Manual	Spiked Average	2.78E+05	2.08E+05	4
	Spiked RSD	8%	7%	
	Blank Average	4.00E+03	1.85E+04	4
	Blank RSD	77%	33%	
Laser-cut	Spiked Average	8.10E+05	7.23E+05	4
	Spiked RSD	10%	20%	
	Blank Average	2.22E+03	1.24E+05	2
	Blank RSD	34%	18%	
Die-cut	Spiked Average	1.14E+06	8.18E+05	4
	Spiked RSD	11%	11%	
	Blank Average	1.15E+03	4.61E+04	3
	Blank RSD	110%	59%	
MDPV				
Cocktail F				
		m/z 175	m/z 135	n
Manual	Spiked Average	1.26E+06	1.12E+06	4
	Spiked RSD	18%	18%	
	Blank Average	1.37E+04	1.33E+04	4
	Blank RSD	40%	47%	
Laser-cut	Spiked Average	2.83E+06	2.66E+06	4
	Spiked RSD	38%	39%	
	Blank Average	1.74E+04	8.56E+04	2
	Blank RSD	28%	10%	
Die-cut	Spiked Average	5.39E+06	4.82E+06	4
	Spiked RSD	22%	23%	
	Blank Average	1.74E+04	5.36E+04	3
	Blank RSD	65%	40%	

Norclozapine				
Cocktail F				
		m/z 192	m/z 270	n
Manual	Spiked Average	3.97E+05	2.93E+05	4
	Spiked RSD	10%	10%	
	Blank Average	5.86E+03	4.94E+03	4
	Blank RSD	53%	52%	
Laser-cut	Spiked Average	6.90E+05	5.11E+05	4
	Spiked RSD	21%	21%	
	Blank Average	7.64E+03	1.12E+04	2
	Blank RSD	16%	67%	
Die-cut	Spiked Average	9.77E+05	7.15E+05	4
	Spiked RSD	14%	15%	
	Blank Average	5.41E+03	8.48E+03	3
	Blank RSD	73%	45%	
Midazolam				
Cocktail F				
		m/z 291	m/z 249	n
Manual	Spiked Average	1.18E+06	3.33E+05	4
	Spiked RSD	10%	10%	
	Blank Average	2.63E+04	7.59E+03	4
	Blank RSD	89%	83%	
Laser-cut	Spiked Average	2.84E+06	7.99E+05	4
	Spiked RSD	38%	37%	
	Blank Average	8.05E+02	2.37E+03	2
	Blank RSD	27%	21%	
Die-cut	Spiked Average	5.40E+06	1.51E+06	4
	Spiked RSD	14%	13%	
	Blank Average	4.86E+02	1.01E+03	3
	Blank RSD	80%	80%	

Labetalol				
Cocktail F				
		m/z 162	m/z 91	n
Manual	Spiked Average	3.31E+05	3.16E+05	4
	Spiked RSD	21%	18%	
	Blank Average	8.10E+03	3.04E+04	4
	Blank RSD	61%	40%	
Laser-cut	Spiked Average	3.22E+05	3.06E+05	4
	Spiked RSD	28%	30%	
	Blank Average	1.34E+04	4.01E+04	2
	Blank RSD	8%	19%	
Die-cut	Spiked Average	3.84E+05	3.58E+05	4
	Spiked RSD	16%	15%	
	Blank Average	7.53E+03	3.84E+04	3
	Blank RSD	63%	84%	
Donepezil				
Cocktail F				
		m/z 91	m/z 243	n
Manual	Spiked Average	1.75E+06	2.81E+05	4
	Spiked RSD	11%	12%	
	Blank Average	2.35E+04	4.56E+03	4
	Blank RSD	48%	44%	
Laser-cut	Spiked Average	6.20E+06	1.01E+06	4
	Spiked RSD	18%	18%	
	Blank Average	2.28E+04	4.06E+03	2
	Blank RSD	6%	11%	
Die-cut	Spiked Average	1.19E+07	1.91E+06	4
	Spiked RSD	12%	12%	
	Blank Average	1.92E+04	2.73E+03	3
	Blank RSD	39%	64%	

Methamphetamine				
Cocktail F				
		m/z 91	m/z 119	n
Manual	Spiked Average	1.14E+06	4.28E+05	4
	Spiked RSD	8%	9%	
	Blank Average	2.21E+04	4.37E+03	4
	Blank RSD	44%	64%	
Laser-cut	Spiked Average	2.28E+06	8.72E+05	4
	Spiked RSD	15%	18%	
	Blank Average	2.16E+04	2.64E+03	2
	Blank RSD	11%	8%	
Die-cut	Spiked Average	4.00E+06	1.53E+06	4
	Spiked RSD	3%	3%	
	Blank Average	5.82E+04	5.50E+03	3
	Blank RSD	55%	98%	

Alpha-PVP				
Cocktail G				
		m/z 91	m/z 77	n
Manual	Spiked Average	1.82E+06	7.53E+05	4
	Spiked RSD	14%	14%	
	Blank Average	3.34E+04	3.16E+04	4
	Blank RSD	104%	61%	
Laser-cut	Spiked Average	2.01E+04	1.08E+05	4
	Spiked RSD	18%	26%	
	Blank Average	2.52E+06	1.07E+06	2
	Blank RSD	25%	23%	
Die-cut	Spiked Average	2.14E+04	7.54E+04	4
	Spiked RSD	51%	52%	
	Blank Average	9.05E+06	3.72E+06	3
	Blank RSD	31%	30%	
Bupropion				
Cocktail G				
		m/z 184	m/z 131	n
Manual	Spiked Average	1.37E+06	7.63E+05	4
	Spiked RSD	11%	10%	
	Blank Average	2.89E+04	1.54E+04	4
	Blank RSD	96%	93%	
Laser-cut	Spiked Average	4.96E+04	2.16E+04	4
	Spiked RSD	35%	20%	
	Blank Average	1.75E+06	9.88E+05	2
	Blank RSD	15%	14%	
Die-cut	Spiked Average	4.66E+04	2.04E+04	4
	Spiked RSD	56%	57%	
	Blank Average	7.20E+06	4.04E+06	3
	Blank RSD	33%	34%	

7-aminoclonazepam				
Cocktail G				
		m/z 121	m/z 94	n
Manual	Spiked Average	1.76E+05	6.92E+04	4
	Spiked RSD	18%	18%	
	Blank Average	9.32E+03	7.98E+03	4
	Blank RSD	61%	53%	
Laser-cut	Spiked Average	3.57E+04	5.48E+04	4
	Spiked RSD	34%	22%	
	Blank Average	7.70E+04	5.21E+04	2
	Blank RSD	26%	31%	
Die-cut	Spiked Average	1.92E+04	2.17E+04	4
	Spiked RSD	51%	68%	
	Blank Average	2.37E+05	1.03E+05	3
	Blank RSD	40%	38%	
Desalkylflurazepam				
Cocktail G				
		m/z 140	m/z 226	n
Manual	Spiked Average	3.19E+05	1.54E+05	4
	Spiked RSD	16%	33%	
	Blank Average	5.43E+04	3.51E+03	4
	Blank RSD	52%	125%	
Laser-cut	Spiked Average	4.50E+03	8.07E+03	4
	Spiked RSD	22%	12%	
	Blank Average	3.69E+04	8.90E+04	2
	Blank RSD	20%	19%	
Die-cut	Spiked Average	1.06E+04	2.16E+03	4
	Spiked RSD	85%	73%	
	Blank Average	1.39E+05	2.08E+05	3
	Blank RSD	21%	26%	

Chlordiazepoxide				
Cocktail G				
		m/z 227	m/z 255	n
Manual	Spiked Average	6.81E+05	1.61E+05	4
	Spiked RSD	11%	12%	
	Blank Average	2.61E+04	9.06E+03	4
	Blank RSD	74%	44%	
Laser-cut	Spiked Average	1.28E+04	1.80E+04	4
	Spiked RSD	24%	2%	
	Blank Average	2.42E+05	6.41E+04	2
	Blank RSD	22%	20%	
Die-cut	Spiked Average	8.80E+03	1.31E+04	4
	Spiked RSD	53%	57%	
	Blank Average	5.17E+05	1.30E+05	3
	Blank RSD	29%	28%	
Cocaethylene				
Cocktail G				
		m/z 196	m/z 82	n
Manual	Spiked Average	3.53E+06	9.50E+05	4
	Spiked RSD	10%	10%	
	Blank Average	5.74E+04	2.28E+04	4
	Blank RSD	103%	79%	
Laser-cut	Spiked Average	1.73E+04	1.27E+04	4
	Spiked RSD	2%	16%	
	Blank Average	6.06E+06	1.62E+06	2
	Blank RSD	19%	20%	
Die-cut	Spiked Average	3.33E+04	8.27E+03	4
	Spiked RSD	84%	57%	
	Blank Average	2.66E+07	7.00E+06	3
	Blank RSD	27%	27%	

Chlorpromazine				
Cocktail G				
		m/z 214	m/z 58	n
Manual	Spiked Average	3.65E+04	1.37E+05	4
	Spiked RSD	10%	11%	
	Blank Average	1.90E+03	6.27E+03	4
	Blank RSD	62%	48%	
Laser-cut	Spiked Average	8.04E+03	6.49E+03	4
	Spiked RSD	12%	27%	
	Blank Average	8.90E+04	3.25E+05	2
	Blank RSD	19%	18%	
Die-cut	Spiked Average	2.15E+03	7.47E+03	4
	Spiked RSD	73%	56%	
	Blank Average	2.08E+05	8.02E+05	3
	Blank RSD	26%	26%	
Clozapine				
Cocktail G				
		m/z 270	m/z 192	n
Manual	Spiked Average	1.32E+06	8.41E+05	4
	Spiked RSD	10%	10%	
	Blank Average	1.72E+04	1.32E+04	4
	Blank RSD	118%	104%	
Laser-cut	Spiked Average	8.55E+03	7.83E+03	4
	Spiked RSD	1%	24%	
	Blank Average	2.61E+06	1.68E+06	2
	Blank RSD	25%	25%	
Die-cut	Spiked Average	3.76E+03	4.60E+03	4
	Spiked RSD	57%	77%	
	Blank Average	6.15E+06	3.97E+06	3
	Blank RSD	36%	37%	

6-acetylmorphine				
Cocktail G				
		m/z 165	m/z 211	n
Manual	Spiked Average	8.69E+04	3.66E+04	4
	Spiked RSD	11%	14%	
	Blank Average	3.76E+04	1.64E+04	4
	Blank RSD	36%	79%	
Laser-cut	Spiked Average	9.03E+03	1.07E+04	4
	Spiked RSD	14%	7%	
	Blank Average	6.61E+04	3.93E+04	2
	Blank RSD	24%	21%	
Die-cut	Spiked Average	1.62E+04	9.08E+03	4
	Spiked RSD	109%	70%	
	Blank Average	9.86E+04	5.12E+04	3
	Blank RSD	26%	25%	
Diltiazem				
Cocktail G				
		m/z 178	m/z 150	n
Manual	Spiked Average	1.83E+06	7.92E+05	4
	Spiked RSD	9%	9%	
	Blank Average	2.40E+04	1.41E+04	4
	Blank RSD	123%	100%	
Laser-cut	Spiked Average	5.97E+03	8.12E+03	4
	Spiked RSD	31%	18%	
	Blank Average	4.91E+06	2.13E+06	2
	Blank RSD	27%	27%	
Die-cut	Spiked Average	2.17E+03	3.92E+03	4
	Spiked RSD	94%	77%	
	Blank Average	1.94E+07	8.48E+06	3
	Blank RSD	31%	32%	

Alfentanil				
Cocktail G				
		m/z 268	m/z 197	n
Manual	Spiked Average	1.71E+05	1.17E+05	4
	Spiked RSD	11%	10%	
	Blank Average	6.35E+03	8.68E+03	4
	Blank RSD	61%	39%	
Laser-cut	Spiked Average	6.27E+02	2.05E+03	4
	Spiked RSD	16%	14%	
	Blank Average	1.99E+05	1.33E+05	2
	Blank RSD	32%	30%	
Die-cut	Spiked Average	3.07E+03	3.79E+03	4
	Spiked RSD	100%	74%	
	Blank Average	1.41E+06	8.76E+05	3
	Blank RSD	42%	39%	

PCP				
Cocktail H				
		m/z 91	m/z 159	n
Manual	Spiked Average	4.76E+05	2.60E+05	4
	Spiked RSD	9%	9%	
	Blank Average	5.58E+04	3.10E+04	4
	Blank RSD	61%	58%	
Laser-cut	Spiked Average	1.37E+06	6.51E+05	4
	Spiked RSD	33%	37%	
	Blank Average	7.27E+04	1.09E+04	2
	Blank RSD	73%	32%	
Die-cut	Spiked Average	1.34E+06	6.85E+05	4
	Spiked RSD	30%	31%	
	Blank Average	4.80E+04	7.39E+03	3
	Blank RSD	39%	50%	
Meperidine				
Cocktail H				
		m/z 220	m/z 174	n
Manual	Spiked Average	8.86E+05	5.04E+05	4
	Spiked RSD	1%	2%	
	Blank Average	5.66E+04	4.36E+04	4
	Blank RSD	94%	73%	
Laser-cut	Spiked Average	2.51E+06	1.55E+06	4
	Spiked RSD	26%	27%	
	Blank Average	8.62E+04	1.14E+05	2
	Blank RSD	59%	67%	
Die-cut	Spiked Average	2.61E+06	1.57E+06	4
	Spiked RSD	28%	31%	
	Blank Average	1.03E+04	1.58E+04	3
	Blank RSD	55%	52%	

Diphenhydramine				
Cocktail H				
		m/z 167	m/z 165	n
Manual	Spiked Average	8.30E+05	3.63E+05	4
	Spiked RSD	2%	1%	
	Blank Average	6.65E+04	2.33E+04	4
	Blank RSD	73%	92%	
Laser-cut	Spiked Average	2.33E+06	1.04E+06	4
	Spiked RSD	28%	30%	
	Blank Average	2.11E+04	1.11E+04	2
	Blank RSD	30%	52%	
Die-cut	Spiked Average	2.49E+06	1.09E+06	4
	Spiked RSD	26%	27%	
	Blank Average	1.25E+04	4.73E+03	3
	Blank RSD	43%	50%	
Doxylamine				
Cocktail H				
		m/z 167	m/z 182	n
Manual	Spiked Average	1.60E+06	1.41E+06	4
	Spiked RSD	7%	8%	
	Blank Average	9.87E+04	7.62E+04	4
	Blank RSD	84%	96%	
Laser-cut	Spiked Average	3.68E+06	3.22E+06	4
	Spiked RSD	37%	34%	
	Blank Average	5.48E+04	3.03E+04	2
	Blank RSD	60%	5%	
Die-cut	Spiked Average	4.15E+06	3.53E+06	4
	Spiked RSD	32%	31%	
	Blank Average	3.29E+04	1.15E+04	3
	Blank RSD	43%	34%	

EDDP				
Cocktail H				
		m/z 234	m/z 249	n
Manual	Spiked Average	2.81E+06	1.15E+06	4
	Spiked RSD	9%	10%	
	Blank Average	2.26E+05	9.19E+04	4
	Blank RSD	118%	118%	
Laser-cut	Spiked Average	1.45E+07	5.95E+06	4
	Spiked RSD	28%	28%	
	Blank Average	2.96E+04	9.61E+03	2
	Blank RSD	54%	54%	
Die-cut	Spiked Average	1.46E+07	6.05E+06	4
	Spiked RSD	30%	31%	
	Blank Average	1.04E+04	2.21E+03	3
	Blank RSD	36%	61%	
Promethazine				
Cocktail H				
		m/z 86	m/z 198	n
Manual	Spiked Average	3.07E+05	1.31E+05	4
	Spiked RSD	1%	1%	
	Blank Average	5.14E+04	2.13E+04	4
	Blank RSD	34%	39%	
Laser-cut	Spiked Average	8.55E+05	3.82E+05	4
	Spiked RSD	30%	32%	
	Blank Average	5.14E+04	3.27E+04	2
	Blank RSD	0%	16%	
Die-cut	Spiked Average	7.36E+05	3.23E+05	4
	Spiked RSD	28%	29%	
	Blank Average	4.40E+04	1.98E+04	3
	Blank RSD	26%	23%	

Brompheniramine				
Cocktail H				
		m/z 274	m/z 167	n
Manual	Spiked Average	7.88E+05	3.90E+05	4
	Spiked RSD	5%	5%	
	Blank Average	3.67E+04	4.20E+04	4
	Blank RSD	93%	38%	
Laser-cut	Spiked Average	2.64E+06	1.29E+06	4
	Spiked RSD	32%	34%	
	Blank Average	1.32E+04	1.31E+04	2
	Blank RSD	69%	77%	
Die-cut	Spiked Average	2.45E+06	1.17E+06	4
	Spiked RSD	27%	28%	
	Blank Average	3.41E+03	4.74E+03	3
	Blank RSD	63%	63%	
Lorazepam				
Cocktail H				
		m/z 276	m/z 167	n
Manual	Spiked Average	7.82E+05	3.80E+05	4
	Spiked RSD	5%	4%	
	Blank Average	3.51E+04	3.46E+04	4
	Blank RSD	95%	47%	
Laser-cut	Spiked Average	2.54E+06	1.20E+06	4
	Spiked RSD	29%	30%	
	Blank Average	1.60E+04	1.08E+04	2
	Blank RSD	43%	66%	
Die-cut	Spiked Average	2.41E+06	1.14E+06	4
	Spiked RSD	26%	26%	
	Blank Average	8.18E+03	4.27E+03	3
	Blank RSD	30%	65%	

Normeperidine				
Cocktail H				
		m/z 160	m/z 56	n
Manual	Spiked Average	5.68E+05	1.30E+05	4
	Spiked RSD	6%	6%	
	Blank Average	5.18E+04	1.41E+04	4
	Blank RSD	70%	76%	
Laser-cut	Spiked Average	1.02E+06	2.08E+05	4
	Spiked RSD	10%	10%	
	Blank Average	1.41E+05	1.51E+04	2
	Blank RSD	53%	64%	
Die-cut	Spiked Average	1.15E+06	2.47E+05	4
	Spiked RSD	17%	17%	
	Blank Average	2.92E+04	3.06E+03	3
	Blank RSD	29%	50%	
Flurazepam				
Cocktail H				
		m/z 315	m/z 317	n
Manual	Spiked Average	9.48E+05	2.14E+05	4
	Spiked RSD	2%	3%	
	Blank Average	5.66E+04	1.79E+04	4
	Blank RSD	106%	69%	
Laser-cut	Spiked Average	4.32E+06	9.91E+05	4
	Spiked RSD	34%	34%	
	Blank Average	5.68E+03	2.06E+04	2
	Blank RSD	68%	87%	
Die-cut	Spiked Average	3.66E+06	8.32E+05	4
	Spiked RSD	34%	32%	
	Blank Average	1.85E+03	1.45E+03	3
	Blank RSD	33%	38%	

Morphine				
Cocktail I				
		m/z 152	m/z 165	n
Manual	Spiked Average	1.53E+04	1.22E+04	4
	Spiked RSD	18%	22%	
	Blank Average	3.64E+03	3.80E+03	4
	Blank RSD	70%	56%	
Laser-cut	Spiked Average	3.70E+04	2.30E+04	4
	Spiked RSD	15%	18%	
	Blank Average	24154.03	10725.94	2
	Blank RSD	62%	70%	
Die-cut	Spiked Average	2.56E+04	1.60E+04	1
	Spiked RSD	-	-	
	Blank Average	6.67E+03	3.31E+03	3
	Blank RSD	50%	67%	
Oxymorphone				
Cocktail I				
		m/z 227	m/z 198	n
Manual	Spiked Average	3.15E+04	2.43E+04	4
	Spiked RSD	9%	11%	
	Blank Average	3.76E+03	2.99E+03	4
	Blank RSD	72%	71%	
Laser-cut	Spiked Average	4.18E+04	4.68E+04	4
	Spiked RSD	16%	19%	
	Blank Average	13437.55	32548.87	2
	Blank RSD	68%	72%	
Die-cut	Spiked Average	3.74E+04	3.75E+04	1
	Spiked RSD	-	-	
	Blank Average	3.06E+03	5.25E+03	3
	Blank RSD	58%	43%	

Zaleplon				
Cocktail I				
		m/z 236	m/z 264	n
Manual	Spiked Average	5.80E+04	5.11E+04	4
	Spiked RSD	41%	41%	
	Blank Average	3.72E+03	3.41E+03	4
	Blank RSD	91%	66%	
Laser-cut	Spiked Average	5.74E+04	3.91E+04	4
	Spiked RSD	31%	25%	
	Blank Average	40471.3	13342.55	2
	Blank RSD	64%	60%	
Die-cut	Spiked Average	6.25E+04	6.21E+04	1
	Spiked RSD	-	-	
	Blank Average	3.55E+03	7.67E+03	3
	Blank RSD	64%	88%	
Methadone				
Cocktail I				
		m/z 265	m/z 105	n
Manual	Spiked Average	1.34E+06	4.41E+05	4
	Spiked RSD	11%	10%	
	Blank Average	3.69E+04	1.89E+04	4
	Blank RSD	148%	111%	
Laser-cut	Spiked Average	3.88E+06	1.26E+06	4
	Spiked RSD	32%	33%	
	Blank Average	15483.3	21467.25	2
	Blank RSD	51%	74%	
Die-cut	Spiked Average	1.11E+07	3.66E+06	1
	Spiked RSD	-	-	
	Blank Average	6.30E+03	1.20E+04	3
	Blank RSD	64%	50%	

Clonazepam				
Cocktail I				
		m/z 270	m/z 214	n
Manual	Spiked Average	6.41E+04	1.88E+04	4
	Spiked RSD	50%	51%	
	Blank Average	1.17E+04	1.90E+03	4
	Blank RSD	52%	70%	
Laser-cut	Spiked Average	5.63E+04	2.50E+04	4
	Spiked RSD	29%	31%	
	Blank Average	71418.77	29487.67	2
	Blank RSD	70%	74%	
Die-cut	Spiked Average	5.33E+04	1.27E+04	1
	Spiked RSD	-	-	
	Blank Average	2.09E+04	3.07E+03	3
	Blank RSD	48%	44%	
Fluvoxamine				
Cocktail I				
		m/z 71	m/z 200	n
Manual	Spiked Average	1.02E+05	2.55E+04	4
	Spiked RSD	4%	9%	
	Blank Average	1.00E+04	6.20E+03	4
	Blank RSD	63%	44%	
Laser-cut	Spiked Average	1.76E+05	4.06E+04	4
	Spiked RSD	9%	8%	
	Blank Average	16634.52	10218.4	2
	Blank RSD	86%	6%	
Die-cut	Spiked Average	3.50E+05	8.35E+04	1
	Spiked RSD	-	-	
	Blank Average	9.53E+03	6.28E+03	3
	Blank RSD	42%	24%	

Paroxetine				
Cocktail I				
		m/z 192	m/z 70	n
Manual	Spiked Average	7.46E+04	4.37E+04	4
	Spiked RSD	5%	2%	
	Blank Average	8.35E+03	6.79E+03	4
	Blank RSD	61%	54%	
Laser-cut	Spiked Average	1.29E+05	6.51E+04	4
	Spiked RSD	11%	12%	
	Blank Average	16409.67	7930.324	2
	Blank RSD	39%	72%	
Die-cut	Spiked Average	1.40E+05	7.93E+04	1
	Spiked RSD	-	-	
	Blank Average	5.33E+03	7.07E+03	3
	Blank RSD	44%	69%	
Buspirone				
Cocktail I				
		m/z 122	m/z 95	n
Manual	Spiked Average	7.62E+05	1.74E+05	4
	Spiked RSD	12%	2%	
	Blank Average	2.09E+04	3.20E+04	4
	Blank RSD	131%	43%	
Laser-cut	Spiked Average	3.14E+06	5.83E+05	4
	Spiked RSD	24%	24%	
	Blank Average	26690.31	38870.77	2
	Blank RSD	68%	69%	
Die-cut	Spiked Average	1.13E+07	2.05E+06	1
	Spiked RSD	-	-	
	Blank Average	5.71E+03	2.26E+04	3
	Blank RSD	58%	48%	

Chlorpheniramine				
Cocktail I				
		m/z 230	m/z 167	n
Manual	Spiked Average	9.67E+05	4.96E+05	4
	Spiked RSD	11%	9%	
	Blank Average	2.23E+04	1.77E+04	4
	Blank RSD	149%	113%	
Laser-cut	Spiked Average	2.48E+06	1.25E+06	4
	Spiked RSD	26%	26%	
	Blank Average	93527.49	13034.49	2
	Blank RSD	83%	73%	
Die-cut	Spiked Average	1.04E+07	5.25E+06	1
	Spiked RSD	-	-	
	Blank Average	8.75E+03	3.96E+03	3
	Blank RSD	45%	63%	
Zopiclone				
Cocktail I				
		m/z 245	m/z 111	n
Manual	Spiked Average	1.40E+05	4.89E+04	4
	Spiked RSD	5%	4%	
	Blank Average	2.80E+04	2.72E+03	4
	Blank RSD	29%	75%	
Laser-cut	Spiked Average	1.25E+05	4.17E+04	4
	Spiked RSD	17%	17%	
	Blank Average	34498.15	3883.349	2
	Blank RSD	34%	28%	
Die-cut	Spiked Average	9.94E+04	3.65E+04	1
	Spiked RSD	-	-	
	Blank Average	1.45E+04	5.05E+03	3
	Blank RSD	33%	80%	

Methylphenidate				
Cocktail J				
		m/z 84	m/z 56	n
Manual	Spiked Average	9.00E+05	1.18E+05	4
	Spiked RSD	7%	7%	
	Blank Average	7.69E+04	1.96E+04	4
	Blank RSD	43%	36%	
Laser-cut	Spiked Average	2.67E+06	3.60E+05	4
	Spiked RSD	25%	20%	
	Blank Average	2.34E+04	7.49E+04	3
	Blank RSD	40%	38%	
Nordoxepin				
Cocktail J				
		m/z 107	m/z 235	n
Manual	Spiked Average	1.06E+05	6.27E+04	4
	Spiked RSD	4%	5%	
	Blank Average	1.27E+04	4.58E+03	4
	Blank RSD	55%	72%	
Laser-cut	Spiked Average	2.73E+05	1.53E+05	4
	Spiked RSD	6%	6%	
	Blank Average	2.64E+04	4.20E+03	3
	Blank RSD	50%	68%	
Desipramine				
Cocktail J				
		m/z 72	m/z 193	n
Manual	Spiked Average	1.54E+05	5.64E+04	4
	Spiked RSD	5%	6%	
	Blank Average	8.80E+03	9.66E+03	4
	Blank RSD	69%	32%	
Laser-cut	Spiked Average	4.34E+05	1.16E+05	4
	Spiked RSD	6%	5%	
	Blank Average	1.74E+03	2.35E+04	3
	Blank RSD	59%	53%	

7-aminoflunitrazepam				
Cocktail J				
		m/z 135	m/z 227	n
Manual	Spiked Average	1.17E+05	5.96E+04	4
	Spiked RSD	11%	6%	
	Blank Average	1.11E+04	4.76E+03	4
	Blank RSD	38%	58%	
Laser-cut	Spiked Average	2.42E+05	1.33E+05	4
	Spiked RSD	24%	26%	
	Blank Average	2.79E+04	1.60E+04	3
	Blank RSD	52%	47%	
Trimipramine				
Cocktail J				
		m/z 193	m/z 208	n
Manual	Spiked Average	5.64E+04	4.11E+04	4
	Spiked RSD	6%	7%	
	Blank Average	6.94E+03	5.44E+03	4
	Blank RSD	47%	31%	
Laser-cut	Spiked Average	1.53E+05	1.53E+05	4
	Spiked RSD	19%	19%	
	Blank Average	6.00E+03	5.99E+03	3
	Blank RSD	51%	51%	
Norfluoxetine				
Cocktail J				
		m/z 134	m/z 30	n
Manual	Spiked Average	1.47E+04	2.81E+03	4
	Spiked RSD	10%	12%	
	Blank Average	5.42E+03	2.70E+02	4
	Blank RSD	28%	84%	
Laser-cut	Spiked Average	1.41E+04	2.64E+03	4
	Spiked RSD	7%	6%	
	Blank Average	3.61E+03	3.47E+01	3
	Blank RSD	44%	73%	

Duloxetine				
Cocktail J				
		m/z 44	m/z 154	n
Manual	Spiked Average	1.16E+04	1.11E+04	4
	Spiked RSD	37%	31%	
	Blank Average	5.94E+03	5.66E+03	4
	Blank RSD	66%	66%	
Laser-cut	Spiked Average	1.70E+04	1.49E+04	4
	Spiked RSD	9%	4%	
	Blank Average	1.21E+03	4.43E+03	3
	Blank RSD	82%	46%	
Benztropine				
Cocktail J				
		m/z 167	m/z 165	n
Manual	Spiked Average	4.06E+05	1.82E+05	4
	Spiked RSD	7%	8%	
	Blank Average	3.03E+04	1.44E+04	4
	Blank RSD	38%	41%	
Laser-cut	Spiked Average	1.97E+06	8.94E+05	4
	Spiked RSD	18%	19%	
	Blank Average	1.01E+04	7.58E+03	3
	Blank RSD	59%	64%	
Fluoxetine				
Cocktail J				
		m/z 44	m/z 148	n
Manual	Spiked Average	4.47E+04	7.32E+03	4
	Spiked RSD	4%	7%	
	Blank Average	3.01E+03	1.26E+03	4
	Blank RSD	70%	12%	
Laser-cut	Spiked Average	1.03E+05	1.79E+04	4
	Spiked RSD	4%	7%	
	Blank Average	4.71E+02	6.06E+03	3
	Blank RSD	61%	49%	

Flunitrazepam				
Cocktail J				
		m/z 268	m/z 239	n
Manual	Spiked Average	8.77E+04	2.80E+04	4
	Spiked RSD	14%	15%	
	Blank Average	2.56E+04	3.91E+03	4
	Blank RSD	47%	56%	
Laser-cut	Spiked Average	1.38E+05	3.16E+04	4
	Spiked RSD	18%	22%	
	Blank Average	5.66E+04	6.22E+03	3
	Blank RSD	56%	54%	
Clomipramine				
Cocktail J				
		m/z 227	m/z 242	n
Manual	Spiked Average	3.20E+04	2.05E+04	4
	Spiked RSD	3%	5%	
	Blank Average	8.74E+03	4.26E+03	4
	Blank RSD	33%	37%	
Laser-cut	Spiked Average	1.11E+05	7.42E+04	4
	Spiked RSD	11%	10%	
	Blank Average	1.09E+04	4.75E+03	3
	Blank RSD	69%	57%	
Triazolam				
Cocktail J				
		m/z 308	m/z 239	n
Manual	Spiked Average	4.48E+04	3.81E+04	4
	Spiked RSD	12%	12%	
	Blank Average	3.35E+03	7.35E+03	4
	Blank RSD	85%	42%	
Laser-cut	Spiked Average	1.83E+05	1.47E+05	4
	Spiked RSD	27%	24%	
	Blank Average	5.43E+02	1.14E+04	3
	Blank RSD	66%	60%	

Amlodipine				
Cocktail J				
		m/z 238	m/z 294	n
Manual	Spiked Average	4.44E+04	3.40E+04	4
	Spiked RSD	3%	1%	
	Blank Average	1.79E+04	1.58E+04	4
	Blank RSD	13%	15%	
Laser-cut	Spiked Average	4.00E+04	3.16E+04	4
	Spiked RSD	8%	12%	
	Blank Average	2.38E+03	1.41E+04	3
	Blank RSD	27%	15%	
mCPP				
Cocktail J				
		m/z 154	m/z 118	n
Manual	Spiked Average	1.27E+05	1.03E+05	4
	Spiked RSD	5%	5%	
	Blank Average	1.98E+04	7.94E+03	4
	Blank RSD	56%	62%	
Laser-cut	Spiked Average	1.53E+05	1.37E+05	4
	Spiked RSD	8%	5%	
	Blank Average	1.99E+04	3.15E+04	3
	Blank RSD	44%	43%	

Cyclobenzaprine				
Cocktail K				
		m/z 215	m/z 231	n
Manual	Spiked Average	1.32E+06	5.16E+05	4
	Spiked RSD	121%	121%	
	Blank Average	2.8E+05	1.1E+05	3
	Blank RSD	53%	51%	
Laser-cut	Spiked Average	6.30E+05	2.64E+05	3
	Spiked RSD	31%	32%	
	Blank Average	4.19E+03	1.13E+04	3
	Blank RSD	65%	55%	
Zolpidem				
Cocktail K				
		m/z 92	m/z 235	n
Manual	Spiked Average	9.54E+05	5.07E+06	4
	Spiked RSD	103%	104%	
	Blank Average	2.1E+05	1.1E+06	3
	Blank RSD	49%	51%	
Laser-cut	Spiked Average	6.59E+05	3.34E+06	3
	Spiked RSD	42%	41%	
	Blank Average	2.69E+04	5.62E+03	3
	Blank RSD	51%	54%	
Alprazolam				
Cocktail K				
		m/z 205	m/z 281	n
Manual	Spiked Average	4.81E+05	5.52E+05	4
	Spiked RSD	78%	80%	
	Blank Average	1.7E+05	1.9E+05	3
	Blank RSD	13%	13%	
Laser-cut	Spiked Average	5.68E+04	7.02E+04	3
	Spiked RSD	64%	61%	
	Blank Average	9.88E+03	1.91E+04	3
	Blank RSD	51%	47%	

Fentanyl				
Cocktail K				
		m/z 188	m/z 105	n
Manual	Spiked Average	2.23E+06	1.70E+06	4
	Spiked RSD	114%	108%	
	Blank Average	4.6E+05	4.0E+05	3
	Blank RSD	54%	43%	
Laser-cut	Spiked Average	1.46E+06	1.10E+06	3
	Spiked RSD	37%	37%	
	Blank Average	1.08E+05	4.19E+04	3
	Blank RSD	19%	37%	
Haloperidol				
Cocktail K				
		m/z 122	m/z 165	n
Manual	Spiked Average	1.30E+06	1.20E+06	4
	Spiked RSD	115%	117%	
	Blank Average	3.1E+05	2.8E+05	3
	Blank RSD	51%	52%	
Laser-cut	Spiked Average	6.23E+05	5.64E+05	3
	Spiked RSD	36%	36%	
	Blank Average	1.60E+04	1.42E+04	3
	Blank RSD	52%	39%	
Risperidone				
Cocktail K				
		m/z 191	m/z 110	n
Manual	Spiked Average	2.45E+06	1.96E+05	4
	Spiked RSD	100%	96%	
	Blank Average	7.3E+05	6.2E+04	3
	Blank RSD	56%	48%	
Laser-cut	Spiked Average	2.11E+06	1.75E+05	3
	Spiked RSD	33%	35%	
	Blank Average	1.50E+04	9.35E+03	3
	Blank RSD	45%	62%	

9-hydroxyrisperidone				
Cocktail K				
		m/z 207	m/z 110	n
Manual	Spiked Average	1.75E+06	3.88E+05	4
	Spiked RSD	82%	84%	
	Blank Average	5.8E+05	1.4E+05	3
	Blank RSD	55%	54%	
Laser-cut	Spiked Average	1.49E+06	3.58E+05	3
	Spiked RSD	36%	38%	
	Blank Average	1.90E+04	2.20E+04	3
	Blank RSD	31%	48%	
Ropinirole				
Cocktail K				
		m/z 114	m/z 132	n
Manual	Spiked Average	1.31E+06	2.90E+05	4
	Spiked RSD	112%	111%	
	Blank Average	2.8E+05	6.5E+04	3
	Blank RSD	53%	49%	
Laser-cut	Spiked Average	5.49E+05	1.72E+05	3
	Spiked RSD	33%	34%	
	Blank Average	7.84E+03	4.82E+04	3
	Blank RSD	35%	49%	
Dextromethorphan				
Cocktail K				
		m/z 215	m/z 147	n
Manual	Spiked Average	8.50E+05	6.58E+05	4
	Spiked RSD	119%	118%	
	Blank Average	1.9E+05	1.5E+05	3
	Blank RSD	52%	52%	
Laser-cut	Spiked Average	4.84E+05	3.93E+05	3
	Spiked RSD	28%	29%	
	Blank Average	2.06E+04	3.54E+04	3
	Blank RSD	50%	50%	

Benzoylecgonine				
Cocktail L				
		m/z 168	m/z 77	n
Manual	Spiked Average	4.30E+05	1.65E+05	4
	Spiked RSD	42%	37%	
	Blank Average	4.05E+04	3.76E+04	4
	Blank RSD	62%	92%	
Laser-cut	Spiked Average	7.02E+05	3.58E+05	4
	Spiked RSD	84%	75%	
	Blank Average	9.14E+03	8.12E+04	4
	Blank RSD	85%	85%	
Amitriptyline				
Cocktail L				
		m/z 233	m/z 117	n
Manual	Spiked Average	2.30E+05	1.42E+05	4
	Spiked RSD	17%	17%	
	Blank Average	1.07E+04	1.01E+04	4
	Blank RSD	67%	77%	
Laser-cut	Spiked Average	7.67E+05	4.51E+05	4
	Spiked RSD	43%	41%	
	Blank Average	2.06E+04	9.81E+03	4
	Blank RSD	53%	59%	
Nortriptyline				
Cocktail L				
		m/z 233	m/z 117	n
Manual	Spiked Average	1.83E+05	8.16E+04	4
	Spiked RSD	16%	12%	
	Blank Average	5.82E+03	6.54E+03	4
	Blank RSD	84%	66%	
Laser-cut	Spiked Average	3.87E+05	1.70E+05	4
	Spiked RSD	18%	14%	
	Blank Average	7.10E+03	7.29E+03	4
	Blank RSD	21%	69%	

Atenolol				
Cocktail L				
		m/z 145	m/z 190	n
Manual	Spiked Average	9.32E+05	6.53E+05	4
	Spiked RSD	18%	19%	
	Blank Average	1.47E+05	5.46E+04	4
	Blank RSD	84%	103%	
Laser-cut	Spiked Average	9.65E+05	7.43E+05	4
	Spiked RSD	3%	4%	
	Blank Average	2.15E+04	4.91E+03	4
	Blank RSD	66%	63%	
Hydrocodone				
Cocktail L				
		m/z 199	m/z 171	n
Manual	Spiked Average	1.87E+05	7.52E+04	4
	Spiked RSD	18%	16%	
	Blank Average	9.96E+03	7.35E+03	4
	Blank RSD	67%	70%	
Laser-cut	Spiked Average	1.57E+05	8.85E+04	4
	Spiked RSD	10%	36%	
	Blank Average	9.32E+03	1.68E+04	4
	Blank RSD	89%	86%	
Diazepam				
Cocktail L				
		m/z 154	m/z 222	n
Manual	Spiked Average	2.53E+05	1.98E+05	4
	Spiked RSD	26%	23%	
	Blank Average	1.40E+04	8.84E+03	4
	Blank RSD	91%	105%	
Laser-cut	Spiked Average	1.67E+05	1.35E+05	4
	Spiked RSD	25%	27%	
	Blank Average	8.90E+03	4.00E+03	4
	Blank RSD	83%	81%	

Temazepam				
Cocktail M				
		m/z 177	m/z 239	n
Manual	Spiked Average	5.10E+04	1.35E+04	4
	Spiked RSD	14%	9%	
	Blank Average	2.44E+04	4.00E+03	4
	Blank RSD	70%	56%	
Laser-cut	Spiked Average	4.33E+04	2.05E+04	4
	Spiked RSD	27%	30%	
	Blank Average	6.43E+03	5.90E+03	4
	Blank RSD	93%	74%	
Doxepine				
Cocktail M				
		m/z 107	m/z 165	n
Manual	Spiked Average	2.08E+05	9.94E+04	4
	Spiked RSD	17%	18%	
	Blank Average	1.76E+04	6.45E+03	4
	Blank RSD	73%	79%	
Laser-cut	Spiked Average	5.21E+05	2.65E+05	4
	Spiked RSD	24%	24%	
	Blank Average	1.83E+04	9.39E+03	4
	Blank RSD	51%	75%	
Nordiazepam				
Cocktail M				
		m/z 140	m/z 208	n
Manual	Spiked Average	2.05E+05	1.47E+05	4
	Spiked RSD	25%	25%	
	Blank Average	4.64E+03	5.02E+03	4
	Blank RSD	29%	46%	
Laser-cut	Spiked Average	8.33E+04	6.05E+04	4
	Spiked RSD	33%	33%	
	Blank Average	3.40E+03	3.71E+03	4
	Blank RSD	99%	75%	

Citalopram				
Cocktail M				
		m/z 262	m/z 234	n
Manual	Spiked Average	3.01E+04	2.35E+04	4
	Spiked RSD	17%	11%	
	Blank Average	1.83E+03	3.90E+03	4
	Blank RSD	63%	58%	
Laser-cut	Spiked Average	7.36E+04	4.99E+04	4
	Spiked RSD	17%	16%	
	Blank Average	2.62E+03	4.18E+03	4
	Blank RSD	73%	49%	
Oxazepam				
Cocktail M				
		m/z 241	m/z 77	n
Manual	Spiked Average	1.49E+05	1.33E+05	4
	Spiked RSD	20%	31%	
	Blank Average	8.73E+04	9.94E+04	4
	Blank RSD	65%	83%	
Laser-cut	Spiked Average	1.24E+05	2.33E+05	4
	Spiked RSD	30%	23%	
	Blank Average	5.67E+04	1.23E+05	4
	Blank RSD	64%	46%	
Hydromorphone				
Cocktail M				
		m/z 185	m/z 157	n
Manual	Spiked Average	4.71E+04	3.63E+04	4
	Spiked RSD	10%	11%	
	Blank Average	7.64E+03	8.64E+03	4
	Blank RSD	42%	61%	
Laser-cut	Spiked Average	7.12E+04	7.50E+04	4
	Spiked RSD	16%	24%	
	Blank Average	1.53E+04	2.84E+04	4
	Blank RSD	67%	66%	

Codeine				
Cocktail M				
		m/z 215	m/z 199	n
Manual	Spiked Average	4.74E+04	3.53E+04	4
	Spiked RSD	14%	20%	
	Blank Average	4.67E+03	9.95E+03	4
	Blank RSD	57%	67%	
Laser-cut	Spiked Average	9.30E+04	4.25E+04	4
	Spiked RSD	24%	21%	
	Blank Average	2.54E+04	9.31E+03	4
	Blank RSD	76%	88%	
Cocaine				
Cocktail M				
		m/z 182	m/z 82	n
Manual	Spiked Average	2.40E+06	5.54E+05	4
	Spiked RSD	18%	18%	
	Blank Average	5.27E+04	3.97E+04	4
	Blank RSD	12%	81%	
Laser-cut	Spiked Average	5.73E+06	1.26E+06	4
	Spiked RSD	40%	40%	
	Blank Average	5.89E+04	2.55E+04	4
	Blank RSD	183%	104%	

APPENDIX F

Exogenous interferences identified for each target analyte using the Human Metabolome Database and DrugBank

Target Analyte	Interference Compound	Formula	Molecular Weight
Acetaminophen	2-Amino-3-methylbenzoate	C8H9NO2	151.1
	Dopamine quinone	C8H9NO2	151.1
	Cathine	C9H13NO	151.1
	Phenylpropanolamine	C9H13NO	151.1
	4-Hydroxyamphetamine	C9H13NO	151.1
	Amantadine	C10H17N	151.1
Alfentanil	Trovafloxacin	C20H15F3N4O3	416.1
	Ramipril	C23H32N2O5	416.2
	Forasartan	C23H28N8	416.2
Alpha-PVP	Paracetamol sulfate	C8H9NO5S	231.0
	Isocarboxazid	C12H13N3O2	231.1
	Fenfluramine	C12H16F3N	231.1
	Dexfenfluramine	C12H16F3N	231.1
	Aminophenazone	C13H17N3O	231.1
Alprazolam	Pinazepam	C18H13ClN2O	308.1
	Fluoxetine glucuronide	C16H16N6O	308.1
	Phenylbutazone	C19H20N2O2	308.2
	8-Hydroxycarteolol	C16H24N2O4	308.2
	Indecainide	C20H24N2O	308.2
	Oxybuprocaine	C17H28N2O3	308.2
Amitriptyline; Venlafaxine, EDDP	DOPA sulfate	C9H11NO7S	277.0
	Azathioprine	C9H7N7O2S	277.0
	Ethylmethylthiambutene	C15H19NS2	277.1
	Entecavir	C12H15N5O3	277.1
	Maprotiline	C20H23N	277.2
	N-Desmethylterbinafine	C20H23N	277.2
	Perhexiline	C19H35N	277.3
Amlodipine	Melatonin glucuronide	C19H24N2O8	408.2
	Tamsulosin	C20H28N2O5S	408.2
Amphetamine	Homocysteine	C4H9NO2S	135.0
	Adenine	C5H5N5	135.1
Aripiprazole	Hydromorphone-3-glucoside	C23H29NO8	447.2
Baclofen	Chloroxine	C9H5Cl2NO	213.0

Target Analyte	Interference Compound	Formula	Molecular Weight
	Carmustine	C5H9Cl2N3O2	213.0
	Droxidopa	C9H11NO5	213.1
	Phenazopyridine	C11H11N5	213.1
	Guanadrel Sulfate	C10H19N3O2	213.1
Benzoylecgonine	Quinethazone	C10H12ClN3O3S	289.0
	Chlophedianol	C17H20ClNO	289.1
	Clofedanol	C17H20ClNO	289.1
	Norcocaine	C16H19NO4	289.1
	Chloropyramine	C16H20ClN3	289.1
	Hydroxylated N-acetyl desmethyl frovatriptan	C15H19N3O3	289.1
	Hyoscyamine	C17H23NO3	289.2
	Donepezil metabolite M4	C17H23NO3	289.2
	Atropine	C17H23NO3	289.2
	Dyclonine	C18H27NO2	289.2
Benztropine; Zolpidem	2-oxobrimonidine	C11H10BrN5O	307.0
	Nitazoxanide	C12H9N3O5S	307.0
	Histamine Phosphate	C5H15N3O8P2	307.0
	Glutathione	C10H17N3O6S	307.1
	Tolnaftate	C19H17NOS	307.1
	Alcaftadine	C19H21N3O	307.2
	Ibopamine	C17H25NO4	307.2
	Hydroxyterbinafine	C21H25NO	307.2
	Betaxolol	C18H29NO3	307.2
	Fingolimod	C19H33NO2	307.3
Benzylpiperazine	Pemoline	C9H8N2O2	176.1
	4-Methylaminorex	C10H12N2O	176.1
	Cotinine	C10H12N2O	176.1
Brompheniramine; Chlorpromazine, Fluvoxamine	Clotiazepam	C16H15ClN2OS	318.1
	fluvoxamino acid	C14H17F3N2O3	318.1
	5-Hydroxyemedastine	C17H26N4O2	318.2
	Glycopyrrolate	C19H28NO3	318.2
	Tridihexethyl	C21H36NO	318.3
Buprenorphine	N-Desmethylrosuvastatin	C21H26FN3O6S	467.2
	4-Hydroxytamoxifen sulfate	C26H29NO5S	467.2
	Tobramycin	C18H37N5O9	467.3
Buprenorphine	Tiropramide	C28H41N3O3	467.3
Bupropion	9-Carboxymethoxymethylguanine	C8H9N5O4	239.1
	Salbutamol	C13H21NO3	239.2
	Moprolol	C13H21NO3	239.2

Target Analyte	Interference Compound	Formula	Molecular Weight
	Isoetharine	C13H21NO3	239.2
	Benzphetamine	C17H21N	239.2
Buspirone	5-hydroxylansoprazole	C16H14F3N3O3S	385.1
	Hydroxylansoprazole	C16H14F3N3O3S	385.1
	Lansoprazole sulfone	C16H14F3N3O3S	385.1
	Phosphatidylserine	C13H24NO10P	385.1
	Nilvadipine	C19H19N3O6	385.1
Carbamazepine	Isosorbide Dinitrate	C6H8N2O8	236.0
	Zileuton	C11H12N2O2S	236.1
	Didanosine	C10H12N4O3	236.1
	Hexobarbital	C12H16N2O3	236.1
	O-Desmethyl-lacosamide	C12H16N2O3	236.1
	Procaine	C13H20N2O2	236.2
Carbamazepine-10,11-epoxide; Phenytoin	Hydroxyzileuton	C11H12N2O3S	252.1
	Zileuton sulfoxide	C11H12N2O3S	252.1
	Oxcarbazepine	C15H12N2O2	252.1
	2-Hydroxycarbamazepine	C15H12N2O2	252.1
	Epoxy-hexobarbital	C12H16N2O4	252.1
	3'-Hydroxyhexobarbital	C12H16N2O4	252.1
	Cimetidine	C10H16N6S	252.1
	Talbutal	C13H20N2O3	252.1
Carisoprodol; Ropinirole	Ifosfamide	C7H15Cl2N2O2P	260.0
	Cyclophosphamide	C7H15Cl2N2O2P	260.0
	Fenspiride	C15H20N2O2	260.2
	Oxymetazoline	C16H24N2O	260.2
Chlordiazepoxide; Metoclopraminde; Codeine; Hydrocodone	Imipenem	C12H17N3O4S	299.1
	N-desalkylpropafenone	C18H21NO3	299.2
	N-depropylpropafenone	C18H21NO3	299.2

Target Analyte	Interference Compound	Formula	Molecular Weight
Chlorpheniramine	4-Ketoifosfamide	C7H13Cl2N2O3P	274.0
	4-Ketocyclophosphamide	C7H13Cl2N2O3P	274.0
	5-Hydroxythalidomide	C13H10N2O5	274.1
	Thalidomide arene oxide	C13H10N2O5	274.1
	cis,trans-5'-Hydroxythalidomide	C13H10N2O5	274.1
	N2-Succinoylarginine	C10H18N4O5	274.1
	Ropivacaine	C17H26N2O	274.2
	5-Methoxy-N,N-diisopropyltryptamine	C17H26N2O	274.2
Citalopram	Dorzolamide	C10H16N2O4S3	324.0
	4-hydroxyalprazolam	C17H13ClN4O	324.1
	Alpha-hydroxyalprazolam	C17H13ClN4O	324.1
	Prazepam	C19H17ClN2O	324.1
	Acetohexamide	C15H20N2O4S	324.1
	Dolasetron	C19H20N2O3	324.1
	Ditazole	C19H20N2O3	324.1
	Valaciclovir	C13H20N6O4	324.2
	Quinidine	C20H24N2O2	324.2
	Quinine	C20H24N2O2	324.2
	Diampromide	C21H28N2O	324.2
Clomipramine; Ranitidine; Delta-9-THC	Clorazepate	C16H11ClN2O3	314.0
	Dantrolene	C14H10N4O5	314.1
	Sulfaphenazole	C15H14N4O2S	314.1
	Pergolide	C19H26N2S	314.2
Clonazepam; Oxycodone	Bromazepam	C14H10BrN3O	315.0
	Efavirenz	C14H9ClF3NO2	315.0
	Chlorprothixene	C18H18ClNS	315.1
	Codeine N-oxide	C18H21NO4	315.1
	Rotigotine	C19H25NOS	315.2
	Alizapride	C16H21N5O2	315.2
	Mitiglinide	C19H25NO3	315.2
	Saxagliptin	C18H25N3O2	315.2
Clozapine	Niclosamide	C13H8Cl2N2O4	326.0
	5-Fluorodeoxyuridine monophosphate	C9H12FN2O8P	326.0
	Hydroxyhexamide	C15H22N2O4S	326.1
	Aceprometazine	C19H22N2OS	326.1
	Acepromazine	C19H22N2OS	326.1
	Ajmaline	C20H26N2O2	326.2

Target Analyte	Interference Compound	Formula	Molecular Weight
Cocaethylene	Nilutamide	C12H10F3N3O4	317.1
	N2-Monodes-methylnizatidine	C11H19N5O2S2	317.1
	Arbutamine	C18H23NO4	317.2
	beta-oxycodol	C18H23NO4	317.2
	Nateglinide	C19H27NO3	317.2
	Tetrabenazine	C19H27NO3	317.2
	Butenafine	C23H27N	317.2
Cocaine	Clofarabine	C10H11ClFN5O3	303.1
	Chlorambucil	C14H19Cl2NO2	303.1
	Flumazenil	C15H14FN3O3	303.1
	Pipemidic acid	C14H17N5O3	303.1
Cocaine	Ezogabine	C16H18FN3O2	303.1
	Phenoxybenzamine	C18H22ClNO	303.1
	Fenoterol	C17H21NO4	303.1
	7-Hydroxyetodolac	C17H21NO4	303.1
	alpha-noroxycodol	C17H21NO4	303.1
	Scopolamine	C17H21NO4	303.1
	Hydromorphinol	C17H21NO4	303.1
	Vildagliptin	C17H25N3O2	303.2
Cyclobenzaprine; MDPV	Iobenguane	C8H10IN3	275.0
	Lorcaserin sulfamate	C11H14ClNO3S	275.0
	4'-hydroxypropanolol	C16H21NO3	275.2
	Physostigmine	C15H21N3O2	275.2
	Alphameprodine	C17H25NO2	275.2
Demoxepam; Oxazepam	norclobazam	C15H11ClN2O2	286.1
	nortemazepam	C15H11ClN2O2	286.1
	3-Hydroxynordiazepam	C15H11ClN2O2	286.1
	Phenytoin dihydrodiol	C15H14N2O4	286.1
	4-Hydroxy tolbutamide	C12H18N2O4S	286.1
	Abacavir	C14H18N6O	286.2
	10-alpha-methoxy-9,10-dihydrolysergol	C17H22N2O2	286.2
Desalkylflurazepam; Bupivacaine	Tetrazepam	C16H17ClN2O	288.1
	Sodium lauryl sulfate	C12H25NaO4S	288.1
Desipramine; Atenolol	Carbamazepine-O-quinone	C15H10N2O3	266.1
	Nevirapine	C15H14N4O	266.1
	Practolol	C14H22N2O3	266.2
	Cyclizine	C18H22N2	266.2

Target Analyte	Interference Compound	Formula	Molecular Weight
Desmethylclomipramine; Temazepam	Tazobactam	C10H12N4O5S	300.1
	Clobazam	C16H13ClN2O2	300.1
	Promazine 5-sulfoxide	C17H20N2OS	300.1
	Chlorcyclizine	C18H21ClN2	300.1
Dextromethorphan	6,7-Dichloro-3-hydroxy-1,5 dihydro-imidazo[2,1-b]quinazolin-2-one	C10H7Cl2N3O2	271.0
	4-hydroxy ketorolac	C15H13NO4	271.1
	Norhydromorphone	C16H17NO3	271.1
	Normorphine	C16H17NO3	271.1
	Bupranolol	C14H22ClNO2	271.1
	Desomorphine	C17H21NO2	271.2
	4-Hydroxyatomoxetine	C17H21NO2	271.2
Diphenhydramine; Lamotrigine	Gallium nitrate	GaN3O9	254.9
	Anagrelide	C10H7Cl2N3O	255.0
Diphenhydramine; Lamotrigine	Sulfathiazole	C9H9N3O2S2	255.0
	Pranoprofen	C15H13NO3	255.1
	Ketorolac	C15H13NO3	255.1
	Ganciclovir	C9H13N5O4	255.1
	Hydroxybupropion	C13H18ClNO2	255.1
	Atomoxetine	C17H21NO	255.2
	N-Demethyl orphenadrine	C17H21NO	255.2
	Tripelennamine	C16H21N3	255.2
Donepezil	Trichlormethiazide	C8H8Cl3N3O4S2	378.9
	6-Thioguanosine monophosphate	C10H14N5O7PS	379.0
	6-Thioguanylic acid	C10H14N5O7PS	379.0
	Droperidol	C22H22FN3O2	379.2
Doxepin	7-Hydroxyticlopidine	C14H14ClNOS	279.0
	Ticlopidine S-oxide	C14H14ClNOS	279.0
	2-Oxoticlopidine	C14H14ClNOS	279.0
	Cidofovir	C8H14N3O6P	279.1
	Oxamniquine	C14H21N3O3	279.2
	E-10-Hydroxynortriptyline	C19H21NO	279.2
	Etamiphylline	C13H21N5O2	279.2
	Sibutramine	C17H26ClN	279.2
Duloxetine	Cisplatin	Cl2H4N2Pt	296.9
	2-Chloroticlopidine	C14H13Cl2NS	297.0
	Albendazole sulfone	C12H15N3O4S	297.1
	Nelarabine	C11H15N5O5	297.1
	mono-isopropyl-disopyramide	C18H23N3O	297.2

Target Analyte	Interference Compound	Formula	Molecular Weight
Ephedrine; Pseudoephedrine	Benzocaine	C9H11NO2	165.1
	L-Phenylalanine	C9H11NO2	165.1
	4-Methoxyamphetamine	C10H15NO	165.1
	4-Hydroxymethamphetamine	C10H15NO	165.1
	Hordenine	C10H15NO	165.1
Etomidate	Apraclonidine	C9H10Cl2N4	244.0
	Ribavirin	C8H12N4O5	244.1
	Azacitidine	C8H12N4O5	244.1
	Biotin	C10H16N2O3S	244.1
	Carbidopa	C10H16N2O5	244.1
Felbamate	Rufinamide	C10H8F2N4O	238.1
Fentanyl	Captopril-cysteine disulfide	C12H20N2O5S2	336.1
	Berberine	C20H18NO4	336.1
	Acebutolol	C18H28N2O4	336.2
	Acetyl-alpha-methylfentanyl	C22H28N2O	336.2
Flecainide; Diltiazem	Miconazole	C18H14Cl4N2O	414.0
	Nafcillin	C21H22N2O5S	414.1
	N-desalkyl delavirdine	C19H22N6O3S	414.1
Fluoxetine; Methadone	Lamivudine-monophosphate	C8H12N3O6PS	309.0
Fluoxetine; Methadone	2,8-bis-Trifluoromethyl-4-quinoline carboxylic acid	C12H5F6NO2	309.0
	3-oxobrimonidine	C11H12BrN5O	309.0
	Hydroxylumiracoxib	C15H13ClFNO3	309.1
	Glycodiazine	C13H15N3O4S	309.1
	Ketotifen	C19H19NOS	309.1
	Metixene	C20H23NS	309.2
	Nadolol	C17H27NO4	309.2
	Metipranolol	C17H27NO4	309.2
	Diphenidol	C21H27NO	309.2
	Dicyclomine	C19H35NO2	309.3
Flurazepam	5'-Hydroxylornoxicam	C13H10ClN3O5S2	387.0
	triazolopyridinone epoxide	C19H22ClN5O2	387.1
	4'-hydroxytrazodone	C19H22ClN5O2	387.1
	Terazosin	C19H25N5O4	387.2
	Tamoxifen N-oxide	C26H29NO2	387.2
	4-Hydroxytamoxifen	C26H29NO2	387.2
	alpha-Hydroxytamoxifen	C26H29NO2	387.2
	3-Hydroxytamoxifen (Droloxifene)	C26H29NO2	387.2
Gabapentin	Metronidazole	C6H9N3O3	171.1
	Rasagiline	C12H13N	171.1

Target Analyte	Interference Compound	Formula	Molecular Weight
Haloperidol	Azidocillin	C16H17N5O4S	375.1
	Tiagabine	C20H25NO2S2	375.1
	Gatifloxacin	C19H22FN3O4	375.2
	Benzylmorphine	C24H25NO3	375.2
Hydroxychloroquine	Almotriptan	C17H25N3O2S	335.2
	Naratriptan	C17H25N3O2S	335.2
Hydroxyzine	Bromhexine	C14H20Br2N2	374.0
	Etoricoxib 1'-N'-oxide	C18H15ClN2O3S	374.0
	6-Hydroxymethyletoricoxib	C18H15ClN2O3S	374.0
	N-Desmethylzopiclone	C16H15ClN6O3	374.1
	omega-hydroxyfinasteride	C22H34N2O3	374.3
Ketamine	2-Amino-5-benzoylbenzimidazole	C14H11N3O	237.1
Labetalol	7-hydroxyolanzapine	C17H20N4OS	328.1
	2-hydroxymethylolanzapine	C17H20N4OS	328.1
	Tiapride	C15H24N2O4S	328.1
Labetalol	Methotrimeprazine	C19H24N2OS	328.2
	7-hydroxygranisetron	C18H24N4O2	328.2
	Stanozolol	C21H32N2O	328.3
Levetiracetam	Propylthiouracil	C7H10N2OS	170.1
Lidocaine	p-Chlorobenzene sulfonyl urea	C7H7ClN2O3S	234.0
	4,4'-methanol-bisbenzonitrile	C15H10N2O	234.1
	4'-hydroxymephenytoin	C12H14N2O3	234.1
	S-4-Hydroxymephenytoin	C12H14N2O3	234.1
	Epirizole	C11H14N4O2	234.1
Lorazepam	Enoxacin	C15H17FN4O3	320.1
mCPP	3,7-Dimethyluric acid	C7H8N4O3	196.1
	1,9-Dimethyluric acid	C7H8N4O3	196.1
	7,9-Dimethyluric acid	C7H8N4O3	196.1
	1,3-Dimethyluric acid	C7H8N4O3	196.1
	1,7-Dimethyluric acid	C7H8N4O3	196.1
MDA	Hippuric acid	C9H9NO3	179.1
	Acetylisoniazid	C8H9N3O2	179.1
	Glucosamine	C6H13NO5	179.1
	Mexiletine	C11H17NO	179.1
	Methylephedrine	C11H17NO	179.1
	Methoxyphenamine	C11H17NO	179.1
	Rimantadine	C12H21N	179.2
	Memantine	C12H21N	179.2

Target Analyte	Interference Compound	Formula	Molecular Weight
MDMA	3-Carbamoyl-2-phenylpropionaldehyde	C10H11NO3	193.1
	4-Anilino-4-oxobutanoic acid	C10H11NO3	193.1
	4-Hydroxy-5-phenyltetrahydro-1,3-oxazin-2-one	C10H11NO3	193.1
Meperidine	Pethidine	C15H21NO2	247.2
	desmethylprodine	C15H21NO2	247.2
	Ketobemidone	C15H21NO2	247.2
Mephedrone	Phenmetrazine	C11H15NO	177.1
	Bethanidine	C10H15N3	177.1
	N-Methylnicotinium	C11H17N2	177.1
Mescaline	2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one	C9H9NO5	211.0
	Milrinone; Milrinone Lactate	C12H9N3O	211.1
	5-Hydroxylorcaserin	C11H14ClNO	211.1
	1-Hydroxylorcaserin	C11H14ClNO	211.1
	7-Hydroxylorcaserin	C11H14ClNO	211.1
	4-(4-chlorophenyl)-4-hydroxypiperidine	C11H14ClNO	211.1
	Methyldopa	C10H13NO4	211.1
Mescaline	3-O-Methyl-a-methyldopa	C10H13NO4	211.1
	Zalcitabine	C9H13N3O3	211.1
	Varenicline	C13H13N3	211.1
	Pramipexole	C10H17N3S	211.1
	Isoprenaline	C11H17NO3	211.1
	Orciprenaline	C11H17NO3	211.1
	Isoproterenol	C11H17NO3	211.1
Metaxalone	N-Acetyl-D-glucosamine	C8H15NO6	221.1
	N,N,O-Tridesmethyl-tramadol	C13H19NO2	221.1
	Procarbazine	C12H19N3O	221.2
	Tapentadol	C14H23NO	221.2
Methamphetamine	NAPQI	C8H7NO2	149.0
	Penicillamine	C5H11NO2S	149.1
	L-Methionine	C5H11NO2S	149.1
	Sevelamer	C6H12ClNO	149.1
	Cathinone	C9H11NO	149.1
	Phentermine	C10H15N	149.1

Target Analyte	Interference Compound	Formula	Molecular Weight
Methocarbamol	Moxonidine	C9H12ClN5O	241.1
	3'-Amino-3'-deoxythimidine	C10H15N3O4	241.1
	Mefenamic acid	C15H15NO2	241.1
	Tetrahydrobiopterin	C9H15N5O3	241.1
	N,N-Didemethyl orphenadrine	C16H19NO	241.1
	N-Desmethyldiphenhydramine	C16H19NO	241.1
Methylone	triazolopropionic acid	C9H9N3O3	207.1
	Isoniazid pyruvate	C9H9N3O3	207.1
	Carbamazepine iminoquinone	C14H9NO	207.1
	N-isopropylterephthalamic acid	C11H13NO3	207.1
	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	C10H13N3O2	207.1
	Ciclopirox	C12H17NO2	207.1
	N-Desmethyl tapentadol	C13H21NO	207.2
Methylphenidate; Normeperidine	Dopamine 3-O-sulfate	C8H11NO5S	233.0
	Dopamine 4-sulfate	C8H11NO5S	233.0
	Lomustine	C9H16ClN3O2	233.1
	N,N-Didesmethyltramadol	C15H23NO	233.2
Metoprolol	Sulfisoxazole	C11H13N3O3S	267.1
	Sulfamoxole	C11H13N3O3S	267.1
	Zidovudine	C10H13N5O4	267.1
	Adenosine	C10H13N5O4	267.1
	Vidarabine	C10H13N5O4	267.1
Metoprolol	4-amino-MX	C12H17N3O4	267.1
	Apomorphine	C17H17NO2	267.1
	Voglibose	C10H21NO7	267.1
Mirtazapine; Nordoxepine	Desacetyl-nitazoxanide	C10H7N3O4S	265.0
Mirtazapine; Nordoxepine	Isoniazid alpha-ketoglutaric acid	C11H11N3O5	265.1
	Albendazole	C12H15N3O2S	265.1
	Streptozocin	C8H15N3O7	265.1
	Thiamine	C12H17N4OS	265.1
	E-10-Hydroxydesmethylnortriptyline	C18H19NO	265.1
	Antazoline	C17H19N3	265.2
	Oxprenolol	C15H23NO3	265.2
	4-Hydroxy-alprenolol	C15H23NO3	265.2
Naproxen; TFMPP	Diazoxide	C8H7ClN2O2S	230.0
	Guanabenz	C8H8Cl2N4	230.0
	Hydralazine pyruvate hydrazone	C11H10N4O2	230.1
	cyclic Melatonin	C13H14N2O2	230.1
	Ibudilast	C14H18N2O	230.1

Target Analyte	Interference Compound	Formula	Molecular Weight
Norbuprenorphine	Desacetylcefotaxime	C14H15N5O6S2	413.0
	Dihydroetorphine	C25H35NO4	413.3
Norclozapine; Olanzapine	Ethopropazine	C19H24N2S	312.2
	Praziquantel	C19H24N2O2	312.2
	Granisetron	C18H24N4O	312.2
	Oseltamivir	C16H28N2O4	312.2
Nordiazepam; Doxylamine	Sulfamethizole	C9H10N4O2S2	270.0
	Leflunomide	C12H9F3N2O2	270.1
	A771726	C12H9F3N2O2	270.1
	10,11-Dihydroxycarbamazepine	C15H14N2O3	270.1
	Tolbutamide	C12H18N2O3S	270.1
	Chloroprocaine	C13H19ClN2O2	270.1
	N-Desmethylpromazine	C16H18N2S	270.1
Norfluoxetine	Chlorothiazide	C7H6ClN3O4S2	294.9
	Meclofenamic acid	C14H11Cl2NO2	295.0
	Diclofenac	C14H11Cl2NO2	295.0
	N4-Acetylsulfamethoxazole	C12H13N3O4S	295.1
	Mebendazole	C16H13N3O3	295.1
	Citalopram aldehyde	C18H14FNO2	295.1
	Nor-ketotifen	C18H17NOS	295.1
	Sumatriptan	C14H21N3O2S	295.1
	(E)-2-hydroxydoxepin	C19H21NO2	295.2
	Doxepin N-oxide	C19H21NO2	295.2
Norfluoxetine	Tertatolol	C16H25NO2S	295.2
	Esmolol	C16H25NO4	295.2
Norketamine	Cerulenin	C12H17NO3	223.1
	Neostigmine	C12H19N2O2	223.1
	2,5-Dimethoxy-4-ethylamphetamine	C13H21NO2	223.2
Norpropoxyphene; Midazolam	Amdinocillin	C15H23N3O3S	325.1
	Ergonovine	C19H23N3O2	325.2
	dinor-Levomethadyl acetate	C21H27NO2	325.2
	Bisoprolol	C18H31NO4	325.2
	Dapiprazole	C19H27N5	325.2
	Tolterodine	C22H31NO	325.2

Target Analyte	Interference Compound	Formula	Molecular Weight
Norsertraline	Brimonidine	C11H10BrN5	291.0
	Orciprenaline-3-O-sulfate	C11H17NO6S	291.1
	Diethylthiambutene	C16H21NS2	291.1
	Desethylchloroquine	C16H22ClN3	291.2
	Cyclopentolate	C17H25NO3	291.2
	Levobunolol	C17H25NO3	291.2
	Terbinafine	C21H25N	291.2
	Penbutolol	C18H29NO2	291.2
Nortramadol	Alendronate	C4H13NO7P2	249.0
	Norepinephrine sulfate	C8H11NO6S	249.0
	Sulfapyridine	C11H11N3O2S	249.1
	Epinastine	C16H15N3	249.1
	Desmethylnortriptyline	C18H19N	249.2
	Tenocyclidine	C15H23NS	249.2
	Alprenolol	C15H23NO2	249.2
	N,O-Didesmethylvenlafaxine	C15H23NO2	249.2
Norvenlafaxine; Nortrityline, Tramaol	Amfecloral	C11H12Cl3N	263.0
	Epinephrine sulfate	C9H13NO6S	263.0
	Ticlopidine	C14H14ClNS	263.1
	Gemcitabine	C9H11F2N3O4	263.1
	Dimethylthiambutene	C14H17NS2	263.1
	desethylzaleplon	C15H13N5	263.1
	Protriptyline	C19H21N	263.2
	demethylmaprotiline	C19H21N	263.2
	2-Ethyl-5-methyl-3,3-diphenyl-1-pyrroline	C19H21N	263.2
	Protriptyline	C19H21N	263.2
	Lisdexamfetamine	C15H25N3O	263.2
Oxymorphone	Carboxybupranolol	C14H20ClNO4	301.1
Oxymorphone	Noroxycodone	C17H19NO4	301.1
	Dihydrocodeine	C18H23NO3	301.2
	Dobutamine	C18H23NO3	301.2
	Trihexyphenidyl	C20H31NO	301.2
Paroxetine	Nitisinone	C14H10F3NO5	329.1
	8-Hydroxyamoxapine	C17H16ClN3O2	329.1
	Dopamine glucuronide	C14H19NO8	329.1
	Cloperastine	C20H24ClNO	329.2
	Trilostane	C20H27NO3	329.2
	N-desethyloxybutynin	C20H27NO3	329.2

Target Analyte	Interference Compound	Formula	Molecular Weight
PCP	Cytarabine	C9H13N3O5	243.1
	Agomelatine	C15H17NO2	243.1
	Frovatriptan	C14H17N3O	243.1
Pregabalin	Pargyline	C11H13N	159.1
Primidone; Meprobamate	Mephenytoin	C12H14N2O2	218.1
	N-Acetylserotonin	C12H14N2O2	218.1
	N-despropyl ropinirole	C13H18N2O	218.1
Promethazine; Diazepam	6-Thioinosinic acid	C10H12N4O4S	284.1
	Mazindol	C16H13ClN2O	284.1
	Phenytoin catechol	C15H12N2O4	284.1
	arabinofuranosylguanine	C10H14N5O5	284.1
	Etozoline	C13H20N2O3S	284.1
	Articaine	C13H20N2O3S	284.1
	Promazine	C17H20N2S	284.1
	Tropicamide	C17H20N2O2	284.2
Propoxyphene; Papaverine; Topiramate	Cefacetrile	C13H13N3O6S	339.1
	Methylhydroxygliclazide	C15H21N3O4S	339.1
	7-Hydroxygliclazide	C15H21N3O4S	339.1
	Alogliptin	C18H21N5O2	339.2
	Methylergonovine	C20H25N3O2	339.2
	Methylergometrine	C20H25N3O2	339.2
	noracymethadol	C22H29NO2	339.2
	nor-Levomethadyl acetate	C22H29NO2	339.2
	Disopyramide	C21H29N3O	339.2
	Hexetidine	C21H45N3	339.4
Propranolol	4-Bromo-2,5-dimethoxyphenethylamine	C10H14BrNO2	259.0
	Mizoribine	C9H13N3O6	259.1
	Lenalidomide	C13H13N3O3	259.1
	Clobenzorex	C16H18ClN	259.1
	norzolmitripan	C14H17N3O2	259.1
Propranolol	Ramelteon	C16H21NO2	259.2
	Primaquine	C15H21N3O	259.2
	Eperisone	C17H25NO	259.2

Target Analyte	Interference Compound	Formula	Molecular Weight
Quetiapine	clofarabind-5'-monophosphate	C10H12ClFN5O6P	383.0
	Ceftizoxime	C13H13N5O5S2	383.0
	Brinzolamide	C12H21N3O5S3	383.1
	Felodipine	C18H19Cl2NO4	383.1
	Pantoprazole	C16H15F2N3O4S	383.1
	Meropenem	C17H25N3O5S	383.2
	Prazosin	C19H21N5O4	383.2
Risperidone	Butoconazole	C19H17Cl3N2S	410.0
	Ceftibuten	C15H14N4O6S2	410.0
Sertraline; Zaleplon	Fenoldopam	C16H16ClNO3	305.1
	Entacapone	C14H15N3O5	305.1
Sildenafil	Vandetanib	C22H24BrFN4O2	474.1
Trazodone	Lornoxicam	C13H10ClN3O4S2	371.0
	Carboplatin	C6H12N2O4Pt	371.0
	Berberine chloride	C20H18ClNO4	371.1
	Camazepam	C19H18ClN3O3	371.1
	Isradipine	C19H21N3O5	371.1
	Tamoxifen	C26H29NO	371.2
Triazolam	5-Fluorouridine monophosphate	C9H12FN2O9P	342.0
	Etizolam	C17H15ClN4S	342.1
	Clozapine N-oxide	C18H19ClN4O	342.1
Trimipramine	Estazolam	C16H11ClN4	294.1
	Sulfacytine	C12H14N4O3S	294.1
	Rosoxacin	C17H14N2O3	294.1
	Aspartame	C14H18N2O5	294.1
	Alosetron	C17H18N4O	294.1
	Proparacaine	C16H26N2O3	294.2
	Dimetacrine	C20H26N2	294.2
Verapamil	Sitaxentan	C18H15ClN2O6S2	454.0
	Cefazolin	C14H14N8O4S3	454.0
	Methotrexate	C20H22N8O5	454.2
Ziprasidone	Zileuton O-glucuronide	C17H20N2O8S	412.1
	O-Deethylated candesartan	C22H16N6O3	412.1
	Cinalukast	C23H28N2O3S	412.2
	Trandolapril-d5 Diketopiperazine	C24H32N2O4	412.2
Zopiclone	Sulfinpyrazone sulfide	C23H20N2O2S	388.1
	Bepotastine	C21H25ClN2O3	388.2

Target Analyte	Interference Compound	Formula	Molecular Weight
Zopiclone	Cetirizine	C21H25ClN2O3	388.2
	Bepotastine	C21H25ClN2O3	388.2
	Nisoldipine	C20H24N2O6	388.2
	Trimethobenzamide	C21H28N2O5	388.2
	Ramiprilat	C21H28N2O5	388.2
10-monohydroxyoxcarbazepine	N-acetyl zonisamide	C10H10N2O4S	254.0
	Acetaminophen cystein	C11H14N2O3S	254.1
	2-Hydroxyfelbamate	C11H14N2O5	254.1
	Dyphylline	C10H14N4O4	254.1
	Nepafenac	C15H14N2O2	254.1
	Thiamylal	C12H18N2O2S	254.1
	Midodrine	C12H18N2O4	254.1
6-acetylmorphine	Diloxanide	C14H11Cl2NO4	327.0
	Acetaminophen glucuronide	C14H17NO8	327.1
	Desethylamodiaquine	C18H18ClN3O	327.1
	Naloxone	C19H21NO4	327.1
	Dimenoxadol	C20H25NO3	327.2
	Butorphanol	C21H29NO2	327.2
	Norelgestromin	C21H29NO2	327.2
	Butorphanol	C21H29NO2	327.2
7-aminoclonazepam; Hydromorphone, Morphine	Cladribine	C10H12ClN5O3	285.1
	Faropenem	C12H15NO5S	285.1
	Letrozole	C17H11N5	285.1
	Probenecid	C13H19NO4S	285.1
	Isothipendyl	C16H19N3S	285.1
	Norcodeine	C17H19NO3	285.1
	Norhydrocodone	C17H19NO3	285.1
	N-Monodesmethyl-rizatriptan	C15H19N5O	285.2
	Mepyramine; Pyrilamine	C17H23N3O	285.2
7-aminoflunitrazepam	Risedronate	C7H11NO7P2	283.0
	Oxtriphylline; Choline theophylline	C12H21N5O3	283.2
	Cadralazine	C12H21N5O3	283.2
	alpha-Hydroxymetoprolol	C15H25NO4	283.2
	Levallorphan	C19H25NO	283.2
	N-Dealkylated tolterodine	C19H25NO	283.2
9-hydroxyrisperidone	Iloperidone	C24H27FN2O4	426.2
	Darifenacin	C28H30N2O2	426.2